Pertussis: new insights in Diagnosis, incidence and clinical manifestations

Versteegh, F.G.A.

2005

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Versteegh, F. G. A. (2005). *Pertussis: new insights in Diagnosis, incidence and clinical manifestations.*

Pertussis: new insights in diagnosis, incidence and clinical manifestations

F.G.A. Versteegh

Pertussis: new insights in diagnosis, incidence and clinical manifestations

Vrije Universiteit

Pertussis: new insights in diagnosis, incidence and clinical manifestations

Academisch Proefschrift

ter verkrijging van de graad van doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. T. Sminia,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op woensdag 29 juni 2005 om 15.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Florens Gerard Adriaan Versteegh

geboren te Utrecht

promotor: prof.dr. J.J. Roord

copromotor: dr. J.F.P. Schellekens

THE DELIVERY OF MEDICAL CARE IS TO DO
AS MUCH NOTHING AS POSSIBLE

Samuel Shem MD: The House of God

Mijn dokter

mijn dokter is
een goede dokter
hij heeft mij niet
zieker gemaakt

Willem Hussem

Maar allerdiepst op Curaçao
Treft mij de taal, de gang, de lach,
De ongedwongen oogopslag
Van iedere man en elke vrouw

Anton van Duinkerken: uit: Gesprek in Punda

voor Machelien,
Frédérique, Jan, Hendt

Table of Contents

Chapter 1

Introduction

Introduction

Pertussis (whooping cough) is a highly contagious acute bacterial disease involving the respiratory tract and is caused mainly by *Bordetella* (*B.*) *pertussis* and to a lesser extent by *B.parapertussis*. It is most severe in young infants. It has a worldwide prevalence and occurs in all age groups.

History

The history of whooping cough starts, according to the literature, with the description by Guillaume de Baillou (1538-1616) [1] of an epidemic in 1578 in France, published for the first time only in 1640 by his nephew.

Was the disease not known before, or known maybe by other names and in different countries by different names?

Kohn [2] suggests that the description of the *Perinthus cough* by Hippocrates (around 400 B.C.) might possibly be whooping cough or a mix with other diseases such as viral respiratory infections. In the Oxford English Dictionary [3] *kinkehost* is mentioned in Reginald's Vita Godrici from around 1190. In the "*Middelnederlandsch woordenboek*" [4] (dictionary of medieval Dutch) it is suggested that *gisschen* might be an early eastern Dutch word for whooping cough, used in the first half of the 14th century. In his History of Pediatrics in the Netherlands [5] van Lieburg refers to the Miracle book of the St Jan's cathedral in 's Hertogenbosch, in the southern part of the Netherlands, in which a pilgrimage is described to the statue of the holy Mary because of the recovery of a boy from *kychoest*, in 1383. Nils Rosen von Rosenstein [6] from Sweden states, not knowing when the disease came to his country, that in France it first appeared in 1414, without giving a source. In Schiller-Lübben's "*Mittelniederdeutsches Wörterbuch*" [7] (dictionary of medieval German) *kinkhoste* is found in a source from 1464. Dodonaeus (1517-1585) in his "*Cruijde boeck*" [8] (book of herbs) already in 1554 describes cures for the *kieckhoest*!

De Baillou called it *quinta*, referring to Hippocrates [1]. *Coqueluche*, the present name in French for whooping cough, was then the common name for influenza [9]. Holmes [10] reports that pertussis was called *chyne-cough* in England as early as 1519. Cherry and Heininger [11] say it was called the *kink* (in Scottish synonymous with fit or paroxysm) and *kindhoest* (a Teutonic word meaning child's cough) in the Middle Ages. Nils Rosen von Rosenstein [6] calls it in his book about pediatric diseases from 1798 *Keichhussten*. In a JAMA editorial [12] names as *tosse canina* (dog's bark, Italy), *Wolfshusten* (howling of wolves) and *Eselshusten* (braying of donkeys)(both from Germany) and *chincough* (boisterous laughter, Old English) are given. In Chinese it is called "*cough of 100 days*" [13].

In Dutch it is called *kinkhoest*, coming from old names as *kinkhôste*, *kichhoest*, *keichhusten*,

as van Esso describes [14]. In the Dictionary of the Dutch Language (Woordenboek der Nederlandsche Taal) [15] the same names are given and others as *kie(c)khoest*, *kijkhoest*, *kikhoest*. Also it refers to Dodonaeus (1517-1585) who called the disease *kich*, or *kinchoest* in a latter edition of his "*Cruydt-Boeck*" from 1608 [16].

Since in these old days there were no possibilities to prove the diagnosis we will never know whether all these diseases then were the same as our whooping cough that, as we know today, is caused by *B.pertussis*, or that they were pertussis-like syndromes, caused by one or more other pathogens [17-23].

Clinical Manifestations

Clinical manifestations of whooping cough may show substantial variation depending on previous vaccination, earlier infection with *B.pertussis*, age or the clinical condition of the patient. The clinical course is divided into three stages. After an incubation period of 5 to 10 days, with an upper limit of 21 days, illness begins with the catarrhal phase. This phase lasts 1 to 2 weeks and is usually characterized by low-grade fever, rhinorrhea and progressive cough.

In the subsequent paroxysmal phase, lasting several weeks, *B.pertussis* causes severe and spasmodic cough episodes with a characteristic whoop, often with cyanosis and vomiting. The patient usually appears normal between attacks. Paroxysmal attacks occur more frequently at night with an average of 15 attacks per 24 hours. During the first 1 or 2 weeks of this stage the attacks increase in frequency, then remain at the same level for 2 to 3 weeks and then gradually decrease. The paroxysmal stage usually lasts 1 to 6 weeks but may persist for up to 10 weeks.

Young infants (under 6 months of age) may not have the strength to have a whoop but they do have paroxysms of coughing. The cough though may be absent and disease may then manifest itself with spells of apnoea [24].

Although pertussis may occur at any age, most cases of serious disease and the majority of fatalities are observed in early infancy. The most important complications in the USA are hospitalization (72.2% in children younger than 6 months, 3.9% for those over 20 years of age), bronchopneumonia (17.3% vs. 3.4%), seizures (2.1% vs. 0.5%), acute encephalopathy (0.5% vs. 0.1%), the latter frequently resulting in death or lifelong brain damage and death (0.5% vs. 0) [25]. Heininger reported in proven pertussis patients in Germany an overall complication rate of 5.8%, pneumonia (29%) being the most frequent complication. In infants <6 months of age, the rate of complications was 23.8% [26].

At the end of the catarrhal phase, a leukocytosis with an absolute and relative lymphocytosis frequently begins reaching its peak at the height of the paroxysmal stage. At this time, the total blood leukocyte levels may resemble those of leukemia ($\geq$ 100,000/mm³), with 60 to 80 %

lymphocytes.

The convalescent phase, the last stage, lasting 1 to 3 weeks, is characterized by a gradual, continuous decline of the cough before the patient returns to normal. However, paroxysms often recur with subsequent respiratory infections for many months after the onset of pertussis. Fever is generally minimal throughout the course of pertussis.

Microbiology

The genus Bordetella contains species of related bacteria with similar morphology, size and staining reactions. To date there are 8 species known of Bordetella: *B.pertussis* [27], *B.parapertussis* [28,29], *B.bronchiseptica* [30], *B.avium* [31] (formerly designated *Alcaligenes faecalis*), *B.hinzii* [32,33] (formerly designated *A.faecalis* type II), *B.holmesii* [34], *B.trematum* [35] and *B.petrii* [36]. *B.pertussis*, *B.parapertussis* and *B.bronchiseptica* are genomically closely related. The first four are respiratory pathogens. *B.pertussis* is an obligate human pathogen. *B.pertussis* was long considered the sole agent of whooping cough. A mild, pertussis-like disease in humans may be caused by *B.parapertussis* and occasionally by *B.bronchiseptica*. *B.parapertussis* appears both in humans and animals. The natural habitat of *B.bronchiseptica* is the respiratory tract of smaller animals such as rabbits, cats and dogs. Human infections with *B.bronchiseptica* are rare and occur only after close contact with carrier animals, no human to human transmission occurs. Most patients with severe disease by *B.bronchiseptica* have an (immuno-) compromised clinical status [37]. *B.avium* and *B.hinzii* are important in birds. *B.hinzii* and *B.holmesii* are found in blood cultures from immune compromised patients. *B.trematum* and *B.petrii* have been recently discovered, *B.trematum* in wounds in humans, *B.petrii* (an anaerobic species) in a bioreactor.

B.pertussis is a small (approximately 0.8 μm by 0.4 μm), rod-shaped, or coccoid, or ovoid Gram-negative bacterium that is encapsulated and does not produce spores. It is a strict aerobe. It is arranged singly or in small groups and is not easily distinguished from Haemophilus species. *B.pertussis* and *B.parapertussis* are nonmotile.

Bacteriological confirmation of suspected whooping cough is often missed, as culturable *B.pertussis* does not seem to persist far beyond the catarrhal stage and in addition requires special growth factors to grow on artificial media.

B.pertussis, the causative agent of pertussis with affinity to the mucosal layers of the human respiratory tract, has different antigenic or biologically active components (see table 1), although their exact chemical structure and location in the bacterial cell are known only in part.

Pathogenesis

Infection results in colonization and rapid multiplication of the bacteria on the mucous membranes of the respiratory tract [38]. It produces a number of virulence factors, which comprise pertussis toxin, adenylate cyclase toxin, filamentous haemagglutinin, fimbriae, tracheal cytotoxin, pertactin and dermonecrotic toxin. The expression of these factors is regulated by the *bvg* locus [39,40]. This system assures that the organism synthesizes components only in response to certain environmental stimuli. Bacteremia does not occur. Studies of the different *B.pertussis* adhesion molecules and toxins and their corresponding biologic activities have yielded plausible explanations for many of the symptoms of whooping cough (table 1). In humans, an initial local peribronchial lymphoid hyperplasia occurs accompanied or followed by necrotizing inflammation and leukocyte infiltration in parts of the larynx, trachea and bronchi. Usually, peribronchiolitis and variable patterns of atelectasis and emphysema also develop. To date, there is no possible explanation for the development of the characteristic paroxysmal coughing in pertussis.

Table 1: Biologically active and antigenic components of *Bordetella pertussis* and possible roles in pathogenesis and immunity. [11,13, 39]

Adenylate cyclase toxin (ACT): An extracytoplasmic enzyme that impairs host immune cell function by elevating the levels of intracellular cAMP; by virtue of its hemolysin function it may contribute to local tissue damage in the respiratory tract.

Bordetella **resistance to killing factor (Brk):** A 32-kd outer-membrane protein. An adhesin that also provides resistance to killing by the host's complement system.

Filamentous hemagglutinin (FHA): A cell surface protein. Promotes attachment to respiratory epithelium. Agglutinates erythrocytes in vivo. Antibodies to FHA protect against respiratory tract challenge but not against intracerebral challenge in mice

Fimbriae: Two serologic types (types 2 and 3). Antibody to specific types causes agglutination of the organism. Organisms may contain fimbriae 2, fimbriae 3, fimbriae 2 and 3, or neither fimbriae 2 nor fimbriae 3. Fimbriae may play a critical role as adhesins.

Heat-labile toxin (also called dermonecrotic toxin): Cytoplasmic protein that causes ischemic necrosis at dermal injection site in laboratory animals. It may contribute to local tissue damage in the respiratory tract.

Lipopolysaccharide (LPS) (endotoxin): An envelope toxin with activities similar to endotoxins of other gram-negative bacteria. A significant cause of reactions to whole-cell pertussis vaccines. Antibody to LPS causes agglutination of the organism. Associated with fever and local reactions in mice.

Pertactin (PRN): A 69-kd outer-membrane protein that is an important adhesin. Adenylate cyclase-

associated. Antibody to pertactin causes agglutination of the organism and protects against respiratory tract challenge in mice.

Pertussis toxin (PT) (also called lymphocytosis-promoting factor): A classic bacterial toxin with an enzymatically active A subunit and a B oligomer-binding protein. PT promotes attachment to respiratory epithelium, sensitization to histamine, elicits lymphocytosis, enhances insulin secretion and stimulates adjuvant and mitogenic activity. It is an extracellular envelope protein. It causes T lymphocyte mitogenesis, stimulates interleukin-4 & IgE production, inhibits phagocytic function of leukocytes and it causes cytopathic effect on Chinese hamster ovary cells. Antibodies to PT protect against respiratory tract and intracerebral challenge in mice

Tracheal colonization factor (TCF): A proline-rich protein that functions predominantly as an adhesin in the trachea.

Tracheal cytotoxin (TCT): A disaccharide-tetrapeptide derived from peptidoglycan. Causes local tissue damage in the respiratory tract and ciliary stasis

Type III secretion system (bscN): Several not yet specified proteins that secrete effector proteins into host cells.

Host Defenses

B.pertussis infection and vaccination induce substantial immunity which usually lasts for several years, although in varying degree. Second infections of adults, usually with atypical symptoms and thus not regularly diagnosed as pertussis, may be more frequent than previously assumed [41,42]. Immunity acquired after infection with *B.pertussis* does not protect against other Bordetella species [43].

Pertussis toxin is assumed to be one essential protective immunogen but numerous findings indicate that other components, such as filamentous hemagglutinin, heat-labile toxin, agglutinogens, outer membrane proteins and adenylate cyclase toxin, may also contribute to immunity after infection or vaccination [44-46]. In addition, it was recently shown that antibodies to pertactin but not to pertussis toxin, fimbriae, or filamentous hemagglutinin, are crucial for phagocytosis of *B.pertussis* [46a]. The immunogenicity of these substances may be significantly increased by the presence of pertussis toxin [47]. This synergism indicates that pertussis toxin could function as an adjuvant to a variety of protective antigens of *B.pertussis*. The defense mechanisms are both nonspecific (local inflammation, increase in macrophage activity and production of interferon) and specific (proliferation of specific B and T cells) [48].

The nature of immunity in whooping cough is, however, incompletely understood. A role of circulating antibody in immunity is indicated by the correlation between protection of human vaccinees and their antibody titers [44-46]. However, effective immunity does not necessarily depend on the presence of protective antibodies and immunity to whooping cough may

therefore be mediated essentially by cellular mechanisms [48,49]. This cell-mediated immunity may be considered the crucial carrier of long-term immunity and titers of specific humoral antibodies may diminish over the years.

Epidemiology

World-wide, *B.pertussis* causes some 20-40 million cases of pertussis per year, 90% of which occur in developing countries and an estimated 200 000-400 000 fatalities each year [11,13,50]. Since the last decade many developed countries experience a re-emergence of pertussis, even countries that have had high vaccination coverage for many years. Because of waning natural-derived and vaccine induced immunity older children and adults are susceptible to infection again. Therefore it is assumed that infection-frequency is probably highest in adolescents and adults and consequently those age groups are the main source of infection for infants [51].

In the Netherlands in 1989-1994 the mean incidence on the basis of notification and serology was 2.4 and 2.3 per 100,000 per year. In 1996 there was a steep increase in notifications (27.3/100,000), positive serology, hospital admissions and even deaths [52-54]. Since then the incidence remained higher than before 1996 (Fig 1).
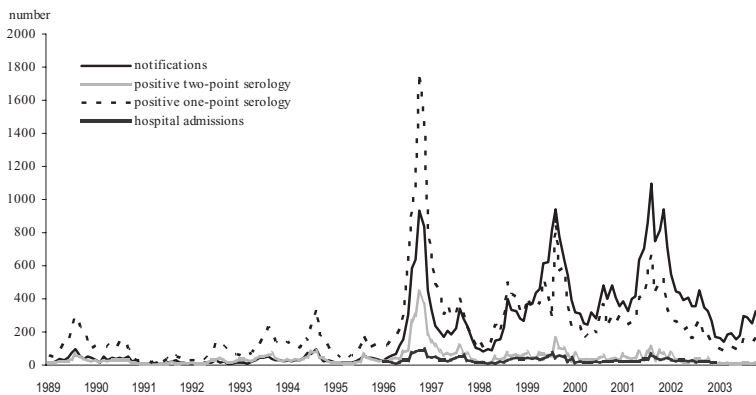
Fig1: Pertussis in the Netherlands from 1989 to 2004: Notifications, positive two point serology, positive one point serology; hospital admissions from 1996 to 2004, based on first day of illness [51]
note: Before 1996 all serological tests for pertussis were performed at the LIS-RIVM. However, since 1998 at least three of the 16 regional Public Health Laboratories and also some other(hospital) laboratories have started to perform serology with commercial available assays. Consequently, the population coverage of serological surveillance based on serological data of LIS-RIVM is now estimated to have decreased from 100% in 1996 to less than 50% in 2002.
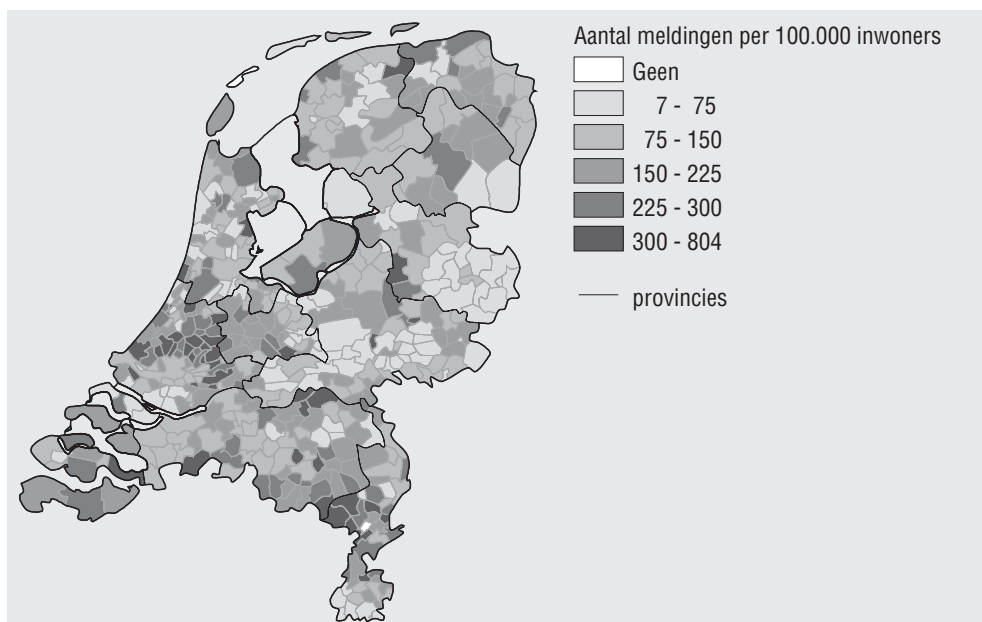
Fig 2: Bordetella pertussis notifications in the Netherlands per city/town from 1-1-2000 to 30-9-2004. Number of notifications per 100.000 inhabitants [94].

Notifications per 100.000 inhabitants vary per city/town from 1-1-2000 to 31-5-2004 from 0 to 610 (Fig 2). Not only there is a strong regional variation in number of notifications but also a discrepancy between notifications and number of positive serology (table 2).

According to the Central Statistics of the Netherlands in 1989-1995 2 deaths due to pertussis were reported. In the period 1996-2002 8 deaths due to pertussis were reported: two in 1996, two in 1997, one in 1998 and three in 1999. For the years 2000, 2001 and 2002 no deaths due to pertussis were reported [51].

Although in older literature there are reports of reinfection with whooping cough, there are no reports on proven reinfection [41,55-59].

Changes in vaccination coverage, vaccine quality or accuracy in reporting have been excluded as possible causes for the increase in incidence. However, there have been adaptations of *B.pertussis* to the vaccine. Notable changes in the variety of *B.pertussis* strains were found between the populations from the prevaccination era and the subsequent period. The reduction in genotypic diversity in the 1960s and 1980s was associated with the expansion of antigenically distinct strains, different from the vaccine strains, showing polymorphism in pertussis toxin and pertactin [60]. This might have contributed to the re-emergence of pertussis in the Netherlands.

Table 2: Incidence and absolute number of pertussis (in table) per year and per source, in the period 1989-2002 [51].

year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Noti-fications	523	397	145	160	346	519	341	4231	2671	2508	6980	4229	8030	4504
Positive two point serology	350	278	110	238	482	498	272	1885	924	468	855	458	701	344
Positive one point serology	1760	1204	411	861	1489	1867	1070	7854	4107	3247	5377	2990	4914	2473
Estimated coverage of serologic data *	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*94%*	*77%*	*72%*	*60%*	*55%*	*51%*
Hospital admissions	221	157	82	101	288	276	162	513	436	282	509	247	397	261

*: Before 1996 all serological tests for pertussis were performed at the LIS-RIVM. However, since 1998 at least three of the 16 regional Public Health Laboratories and also some other (hospital) laboratories have started to perform serology with commercial available assays. Consequently, the population coverage of serological surveillance based on serological data of LIS-RIVM is now estimated to have decreased from 100% in 1996 to less than 50% in 2002.

Transmission

In most countries *B.pertussis* is endemic with superimposed epidemic cycles. These cycles occur approximately every 4 years in vaccinated populations and approximately every 2 to 3 years in non vaccinated populations, although in the Netherlands the incidence is higher now (every 2-3 years) compared to the period prior to the epidemic in 1996-1997 (every 4 years) [51,61]. Most infections occur from July to October. Pertussis is very contagious. It is transmitted obligatory from human to human by direct contact with discharges from respiratory mucous membranes of infected persons primarily via droplets by the airborne route. The mucous membranes of the human respiratory tract are the natural habitat for *B.pertussis* and *B.parapertussis*. Most infections occur after direct contact with diseased persons, specifically by inhalation of bacteria-bearing droplets expelled in cough spray. The patient is most infectious during the early catarrhal phase, when clinical symptoms are relatively mild and noncharacteristic. Subclinical cases may have similar epidemiologic significance. Healthy transient carriers of *B.pertussis* or *B.parapertussis* are assumed to play no significant epidemiologic role. Chronic carriage by humans is not documented.

Immunity

Pertussis infection or vaccination results in a long-lasting but not necessarily lifelong protection

against the typical clinical manifestations of the disease, or reinfection. The protection may not be complete, as atypical or unrecognized infection in presumably immune persons, particularly adults, may be easily overlooked. Also, newborn babies of mothers who have had pertussis are not necessarily protected. Hence, following previous infection, occasional exposure to *B.pertussis* strains circulating in the community may be required to sustain high-level immunity. Although the level of antibodies to pertussis toxin, pertactin or filamentous hemagglutinin are sometimes used as serological indicators of protection [44,45], lack of generally accepted correlates of immunity and animal models are impediments to the evaluation of new pertussis vaccine candidates and the monitoring of the consistency of production.

Although vaccination has caused a firm decrease in incidence and mortality over the years, occasional local epidemics do occur. The disease is especially dangerous in the first 6 months of life. There seems no distinct influence of the season or climate on the morbidity rate but there may be an increase in the summer and fall. Older persons (i.e., adolescents and adults) and those partially protected by the vaccine may become infected with *B.pertussis* but usually have milder or asymptomatic disease [62]. Pertussis in these persons may present as a persistent (>7 days) cough and may be indistinguishable from other upper respiratory infections. Inspiratory whoop is uncommon. In some studies, evidence for a *B.pertussis* infection was found in 25% or more of adults with cough illness lasting >7 days [63,64]. Even though the disease may be milder in older persons, these infected persons may transmit the disease to other susceptible persons, including unimmunized or under immunized infants. Adults are often found to be the first case in a household with multiple pertussis cases [65,66].

Diagnosis

Whooping cough is a clinical diagnosis according to WHO criteria [67] as established in 2000: a case diagnosed by a physician, or a person with a cough lasting at least 2 weeks with at least one of the following symptoms: paroxysms (i.e. fits) of coughing, inspiratory "whooping" or post-tussive vomiting (i.e. vomiting immediately after coughing) without other apparent cause. Criteria for laboratory confirmation are: isolation of *B.pertussis* or detection of genomic sequences by polymerase chain reaction (PCR) or positive paired serology (i.e. fourfold increase) [68].

Only since Bordet and Gengou [27] in 1906 cultured *B.pertussis* we could be sure about the diagnosis. Recovery of *B.pertussis* is the golden standard but culturing *B.pertussis* is not very easy. Bordetellae can be cultured from nasopharyngeal swabs or nasopharyngeal secretions. The sensitivity of the culture depends mainly on the technique of taking the nasopharyngeal swabs (calcium alginate or Dacron) or secretions, direct inoculation of nasopharyngeal swab material onto special freshly prepared media (Bordet-Gengou or Regan-Lowe) for primary isolation and immediate aerobic incubation in a stove. *B.pertussis* grows slowly, thus it is

recommended to extent incubation time from 7 to 14 days.

The newest test for detection of *B.pertussis* and *B.parapertussis* is by polymerase chain reaction (PCR), a very specific test and more sensitive than culture. Nasopharyngeal swabs are suspended, then incubated and amplificated. The final PCR product is analyzed by gel electrophoresis and hybridization. In later stages of the disease PCR testing is more often positive than culture, as in patients treated with antibiotics or in vaccinated patients [69]. The PCR yield is about 2.4 fold higher than culture. The poor performance of culture may be due the fastidious nature of *B.pertussis* but it is also possible that by PCR *B.pertussis* DNA is detected in samples in which the organisms have become nonviable. In patients with clinical symptoms of *B.pertussis* infection and positive serology sensitivity of PCR and culture is low (21% and 7% respectively) but specificity of both is 98% [69].

In adolescents and adults culture or PCR is not useful when disease duration is longer than 3-4 weeks [69]. In contrast, in non vaccinated or partially vaccinated young children culture or PCR is useful in any stage of the disease since they have an immature mucosal immune response and therefore a slower eradication of the bacteria.

Where with increasing disease duration the usefulness of PCR and culture declines thus serology becomes more important. Already in 1911 Bordet and Gengou published the first serological methods, detecting agglutinating antibodies to whole *B.pertussis* cells [70]. This remained the hallmark of pertussis serology for more than 70 years [71]. In the eighties of the 20th century various enzyme immunoassays have been developed and presently immunoglobulin G against pertussis toxin (IgG-PT) is the most used and validated test to prove *B.pertussis* infection [68,71]. IgG-PT is only produced after infection with *B.pertussis*, not by other Bordetella species, nor are any cross reactions described. Although, van der Zee et al showed in 2 PCR-proven cases of *B.parapertussis* infection an significant increase of IgG-PT, suggesting that yet in some strains of *B.parapertussis* the transcriptionally silent IgG-PT genes may have come to expression but a co-infection with *B.pertussis* could not be excluded [69].

In newborns and after vaccination against *B.pertussis,* IgG-PT should be looked at with caution because of transplacental transfer or induction by vaccination [68]. After the fourth vaccination with the Dutch vaccine there is only a temporary and small increase and in both instances there is a fast decrease [72]. However, other whole-cell vaccines and acellular vaccines might induce higher IgG-PT levels [45,73]. Antibodies against other antigens as pertactin, fimbriae and filamentous hemagglutinin (FHA) are also available but less validated and are also produced in reaction to infections with other Bordetella species and perhaps other related bacteria. IgG-PT, appearing as late as week 3 of illness, reaches its peak approximately 4.5 weeks after infection but is retarded in very young children (<1 year). Serology for *B.pertussis* is considered positive by the finding of a significant, ≥ 4-fold increase of IgG antibodies to pertussis toxin (IgG-PT) in paired sera to a level of at least 20 U/ml. This hampers the diagnosis of *B.pertussis* infection, since many patients present themselves later in their disease having already high

levels of IgG-PT without showing a significant increase.

Differential diagnosis

Especially in immunized people or in those who suffered earlier from pertussis infection the atypical complaints may be difficult to distinguish from infection by other pathogens, like adenovirus, influenzavirus, parainfluenza viruses, respiratory syncytial virus, *Chlamydia pneumoniae* or *Mycoplasma pneumoniae*, which may cause a pertussis like syndrome [17-20,23]. Also mixed infections may complicate the diagnosis [21,22].

Treatment and Prevention

Although immunization against *B.pertussis* infection has caused a great reduction in the incidence of pertussis, outbreaks still occur, even in countries with high vaccination coverage. Erythromycin, 40-50 mg/kg/day for 10-14 days, usually considered the treatment of choice, will eliminate viable *B.pertussis* organisms from the respiratory tract within a few days[11,74-77]. A 7 day course of erythromycin has proven to be as efficacious as 14 days [78]. Newer macrolides as azithromycin, 10 mg/kg/day for 3 or 5 days [75,79], or 10 mg/kg the first day and 5 mg/kg/day for 4 days [77,79], or clarithromycin, 10-15 mg/kg/day for 7 days [75,76] have also shown to be effective in the treatment of pertussis with fewer side effects than erythromycin. Erythromycin-resistant strains of *B.pertussis* have been isolated but this seems to be very uncommon [80,81]. Although rare, the use of erythromycin in young infants is associated with hypertrophic pyloric stenosis [82,83]. An alternative to erythromycin is trimethoprim-sulfamethoxazole, 6-10 mg trimethoprim/kg/day for 14 days [84]. Fluoroquinolones have good in vitro activity against both *B.pertussis* and *B.parapertussis* and may be useful in the treatment of *B.pertussis* infection, although there are no supporting clinical data at present [85].

Human hyperimmune pertussis globulin is still used occasionally [86,87]. Further treatment is symptomatic. High altitude or flying has been suggested as an effective treatment for the coughing complaints [88,89].

During the paroxysmal phase of the disease, eradication of the bacteria by antimicrobial drugs, such as erythromycin, will not significantly change the clinical course, although there is some clinical evidence that some macrolides might reduce coughing complaints [90].

Although it is better for susceptible children (unimmunized children without a history of whooping cough) to avoid contact with pertussis patients during the first 4 weeks of their illness, this is often difficult to achieve. Exposed unimmunized children are given a macrolide for 10 days after contact is discontinued or after the patient ceases to be contagious. Exposed immunized children younger than 4 years are most probably protected but protection may be enhanced by macrolides or by a booster dose of acellular pertussis vaccine [91].

Vaccination

Currently, approximately 80% of the world's children are vaccinated against pertussis, most of whom have received the diphtheria-tetanus-whole cell pertussis combination [50].

Pertussis vaccine is produced from smooth forms (phase I) of the bacteria as a killed whole-cell vaccine. General vaccination was introduced in the Netherlands in 1952. Furthermore, since 1 January 1999 the primary vaccination for pertussis has been advanced. From that time children are vaccinated at the age of 2, 3, 4 and 11 months, instead of 3, 4, 5 and 11 months. Finally, in November 2001 a booster vaccination with an acellular vaccine, comprised of pertussis toxin, pertactin and filamentous hemagglutinin, was introduced in the National Immunisation Programme at the age of 4 years [51].

Owing to a relatively mild course of disease and to occasional complications after vaccination, it has been argued that general vaccination with the whole-cell vaccine is no longer justified. Therefore acellular pertussis vaccines have been developed. These vaccines are composed very differently and contain various amounts of structural components from the bacteria. Components available for vaccine production include pertussis toxin (which is detoxified), filamentous hemagglutinin, pertactin and fimbrial antigens 2 and 3. Since 2000 4 year old children in the Netherlands are given a booster with acellular vaccine. Recent data suggest that after primary vaccinations of infants these vaccines can convey similar levels of protection as the whole-cell vaccine. Thus, acellular vaccines have also been licensed for primary vaccination. In the Netherlands this acellular vaccine will be introduced for regular vaccination in 2005 [51,91,92].

Aim and outline of this thesis:

In 1996 there was an outbreak of pertussis in the Netherlands, both in vaccinated and in non vaccinated people of all ages. Many questions arose what the cause or causes for this sudden increase in *B.pertussis* infection were. *Among others the question arose whether vaccination or previous natural infection with B.pertussis guaranteed lifelong protection*. As stated before criteria for laboratory confirmation of *B.pertussis* infection are: isolation of *B.pertussis* or detection of genomic sequences by PCR or significant, ≥ 4-fold increase of IgG-PT in paired sera to a level of at least 20 U/ml. Because many patients visit their physician after weeks of coughing, culture or PCR are less sensitive and serology may already show high levels of IgG-PT without significant increase anymore. *Thus we wondered if one point serology could be a useful tool in the diagnosis of B.pertussis infection.* Since there are no cutoff values in one point serology as proof of actual or recent *B.pertussis* infection it would be opportune to develop such cutoff values. Therefore it is important to establish what level of IgG-PT is proof of a

recent *B.pertussis* infection. *Consequently we wondered what the natural course of IgG-PT is after infection.* Accordingly it is necessary to gain insight in the rise, peak and decline of IgG-PT after natural infection with *B.pertussis*. Since *B.pertussis* infection is especially dangerous in young children not or partially vaccinated and since the main source of infection for these children are adults with often atypical clinical manifestations, it is important to gain insight in the incidence of *B.pertussis* infection [93]. *Is it possible, once knowing the natural course of IgG-PT, to calculate the incidence of B.pertussis infection in the Netherlands in different age groups from available surveillance data on IgG-PT levels in the general population?*

For these reasons we investigated the level of IgG-PT in patients who suffered from *B.pertussis* infection, in the years after their infection, every time a blood sample was taken for other reasons. Following these patients through the years we looked out for patients with renewed clinical symptoms of *B.pertussis* infection and a renewed increase of IgG-PT. From other studies [17-22,24] it is known that in some patients there may be evidence of other pathogens involved in the pertussis syndrome besides *B.pertussis* and in others of pertussis-like complaints without proof of *B.pertussis* infection. *We questioned therefore whether there are many mixed infections in patients with pertussis like complaints and which pathogens are involved.*

The aim of this thesis is to find an answer to the following questions:

1: Which titer of IgG-PT in one point serology is proof for recent infection?

2: May a patient suffer from *B.pertussis* infection more than once in a lifetime?

3: What is the natural course of antibodies against *B.pertussis* after infection?

4: What is the yearly incidence of *B.pertussis* infection?

5: What is the best way to protect newborns and not yet (fully) vaccinated babies against *B.pertussis* infection?

6: How often mixed infections occur in *B.pertussis* infection?

7: What is the role of other respiratory pathogens in the pertussis like syndrome?

References

1: Baillou G: Constitutio aestiva. In: RH Major: Classic description of disease. Charles C Thomas, Springfield Ill. 1965 3rd ed, 6th print, pp 210-212

2: Kohn GC: Cough of Perinthus. In: The Wordsworth enceclopedia of plague and pestilence, Wordsworth edition Ltd, Hertfordshire 1998, p 66

3: Oxford English Dictionary. 2nd ed. 1989 (ed. J.A.Simpson and E.S.C.Weiner), Additions 1993-7 and 3rd ed. (in progress) March 2000- (ed. John Simpson). OED Online. Oxford University Press. http://oed.com

4: *Middelnederlandsch Woordenboek op cd-rom*. 's-Gravenhage: Sdu Uitgevers, 1998 (E.Verwijs en J.Verdam, *Middelnederlandsch Woordenboek*. Delen I-IX. 's-Gravenhage: Martinus Nijhoff 1885-1929)

5: Lieburg MJ van: De geschiedenis van de kindergeneeskunde in Nederland. pp 25 and 159. Erasmus Publishing, Rotterdam 1997

6: Rosén von Rosenstein N: von dem Keichhusten. In: Anweisung zur Konntiß und Kur der Kinderkrankheiten. JC Dieterich, Göttingen 1798, pp 417-439

7: Schiller K, Lübben A: *Mittelniederdeutsches Wörterbuch*. Delen 1-6. Wiesbaden/Münster: Dr. Martin Sändig iHG/Aschendorffsche Verlagsbuchhandlung, 1875-1881. (Fotomech. herdruk 1969)

8: Dodoens (= Dodonaeus) R: Cruijde boeck. In den welcken die gheheele historie (...) van den Cruyden (...) met grooter neersticheyt begrepen ende verclaert es (...). Antwerpen, 1554.

9: Goodall EW: A french epidemiologist of the sixteenth century. In: Annals of Medical History 1935;VII;5:409-427

10: Holmes WH: Bacillary and rickettsial infections: acute and chronic, black death to white plague. New York, Macmillan, 1940; 395-414

11: Cherry JD, Heininger U: Pertussis and other Bordetella infections. In: Textbook of Pediatric Infectious Diseases. Eds. Feigin RD, Cherry JD, Demmler GJ, Kaplan SL 1588-1608 Saunders Philadelphia 2004

12: [No authors listed]: Pertussis. JAMA 1967;202:357-358

13: Long SS: *Bordetella pertussis* (Pertussis) and other species. In: Pediatric Infectious Diseases. Eds. Long, Pickering, Prober, 976-986, Churchill Livingstone, New York 1997

14: Esso I van: De etymologie der woorden "kinkhoest", "mazelen", "roode hond", "bof", "quinte de toux" en "coqueluche". Ned Tijdschr Geneeskd 1936;80 III:3565-3566

15: *Het Woordenboek der Nederlandsche Taal op CD-Rom*. (Delen I-XXIX met *Supplementdeel* en *Aanvullingen*). 's-Gravenhage: Sdu Uitgevers, 2003

(M. de Vries, L.A. te Winkel e.a., *Woordenboek der Nederlandsche Taal*. Delen I-XXIX met Supplementdeel. 's-Gravenhage/Leiden etc.: M. Nijhoff/A.W. Sijthoff etc., 1882-1998. Aanvullingen

delen I-III. 's-Gravenhage: Sdu Uitgevers, 2001)

16: Dodonaeus R: Herbarius oft Cruydt-Boeck. Leiden, 1608

17: Connor JD: Evidence for an etiologic role of adenoviral infection in pertussis syndrome. NEJM 1970;283: 390-394

18: Sturdy PM, Court SDM, Gardner PS: Viruses and whooping-cough. Lancet 1971;2(7731):978-979

19: Nelson KE, Gavitt F, Batt MD, Kallick CA, Reddi KT, Levin S: The role of adenoviruses in the pertussis syndrome. J Pediatr 1975;86:335-341

20: Wirsing von König CH, Rott H, Bogaerts H, Schmitt HJ: A serological study of organisms possibly associated with pertussis-like coughing. Pediatr Infect Dis J 1998;17:645-649

21: Tristram DA, Miller RW, McMillan JA, Weiner LB: Simultaneous infection with respiratory syncytial virus and other respiratory pathogens, Am J Dis Child 1988;12:834-836

22: Aoyama T, Ide Y, Watanabe J, Takeuchi Y, Imaizumi A: Respiratory failure caused by dual infection with *Bordetella pertussis* and respiratory syncytial virus. Acta Paediat Japon 1996;38:282-285

23: Hagiwara K, Ouchi K, Tashiro N, Azuma M, Kobayashi K: An epidemic of a pertussis-like illness caused by *Chlamydia pneumoniae*. Pediatr Infect Dis J 1999;18:271-275

24: Christie CDC, Baltimore RS: Pertussis in neonates. Am J Dis Child 1989;143:1199-1202

25: Güriş D, Strebel PM, Bardenheier B, Brennan M, Tachdjian R, Finch E, Wharton M, Livengood JR: Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990-1996. Clin Infect Dis 1999;28:1230-1237

26: Heininger U, Klich K, Stehr K, Cherry JD: Clinical findings in *Bordetella pertussis* infections: results of a prospective multicenter surveillance study. Pediatrics. 1997;100:E10

27: Bordet J, Gengou O: Le microbe de la coqueluche. Ann Inst Pasteur 1906;20:731-741

28: Bradford WL, Slavin B: An organism resembling *Hemophilus pertussis*, with special reference to color changes produced by its growth upon certain media. Am. J. Public Health 1937;27:1277-1282

29: Eldering G, Kendrick PL: *Bacillus parapertussis*: A species resembling both *Bacillus pertussis* and *Bacillus bronchisepticus* but identical with neither. J Bacteriol 1938;35:561-572

30: Ferry NS: *Bacillus bronchisepticus* (bronchicanis): the cause of distemper in dogs and a similar disease in other animals. Veterinary J 1912;68:376-391

31: Kersters K, Hinz KH, 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

32: Cookson BT, Vandamme P, Carlson LC, Larson AM, Sheffield JV, Kersters K, Spach DH: Bacteremia caused by a novel Bordetella species, "*B. hinzii*". J Clin Microbiol. 1994;32:2569-2571

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

34: Weyant RS, Hollis DG, Weaver RE, Amin MF, Steigerwalt AG, O'Connor SP, Whitney AM, Daneshvar MI, Moss CW, Brenner DJ: *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. J Clin Microbiol 1995;33:1-7

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

36: Wintzingerode F von, Schattke A, Siddiqui RA, Rosick U, Gobel UB, Gross R: *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor and emended description of the genus Bordetella. Int J Syst Evol Microbiol 2001;51(Pt 4):1257-1265

37: Woolfrey BF, Moody JA: Human infections associated with *Bordetella bronchiseptica.* Clin Microbiol Rev 1991;4:243-255

38: Finger H, Wirsing von Koenig CH: Bordetella. In: Baron S (ed), Medical Microbiology, pp 403-412, The University of Texas Medical Branch, Galveston 1996

39: Hewlett EL: Pertussis: current concepts of pathogenesis and prevention. Ped Infect Dis J 1997;16: S78-84

40: Madan Babu M, Bhargavi J, Ranajeet Singh Saund, Kumar Singh S: Virulence factors of *Bordetella pertussis*. Curr Science 2001;80:1512-1522

41: Laing JS, Hay M: Whooping-cough: its prevalence and mortality in Aberdeen. Public Health 1902;14:584-598

42: Cherry JD: Pertussis in the preantibiotic and prevaccine era, with emphasis on adult pertussis. Clin Infect Dis 1999;28 (Suppl 2):S107-111

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

44: Cherry JD, Gornbein J, Heininger U, Stehr K: A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. Vaccine 1998;16:1901-1906

45: Storsaeter J, Hallander HO, Gustafsson L, Olin P: Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine 1998;16:1907-1916

46: Storsaeter J, Hallander HO, Gustafsson L, Olin P: Low levels of antipertussis antibodies plus lack of history of pertussis correlate with susceptibility after household exposure to *Bordetella pertussis*. Vaccine 2003;21:3542-3549

46a:Hellwig SMM, Rodriguez ME, Berbers GAM, Winkel JGJ van de, Mooi FR: Crucial role of antibodies to pertactin in *Bordetella pertussis* immunity. J Infect Dis 2003;188:738-742

47: Tonon S, Goriely S, Aksoy E, Pradier O, Del Giudice G, Trannoy E, Willems F, Goldman M, Wit D de: *Bordetella pertussis* toxin induces the release of inflammatory cytokines and dendritic cell activation in whole blood: impaired responses in human newborns. Eur J Immunol 2002;32:3118-3125

48: Ausiello CM, Lande R, Urbani F, Di Carlo B, Stefanelli P, Salmaso S, Mastrantonio P, Cassone A: Cell-mediated immunity and antibody responses to *Bordetella pertussis* antigens in children with a history of pertussis infection and in recipients of an acellular pertussis vaccine. J Infect Dis 2000;181:1989-1995

49: Tran Minh NN, He Q, Edelman K, Olander RM, Viljanen MK, Arvilommi H, Mertsola J: Cell-

mediated immune responses to antigens of *Bordetella pertussis* and protection against pertussis in school children. Pediatr Infect Dis J 1999;18:366-370

50: WHO-Weekly Epidemiological Record, 1999, 74 (18):137-142

51: Greeff SC de, Schellekens JFP, Mooi FR, Melker HE de: Pertussis in the Netherlands 2001-2002. RIVM rapport 128507010/2003

52: Melker HE de, Conyn-van Spaendonck MAE, Rumke HC, Wijngaarden JK van , Mooi FR, Schellekens JFP: Pertussis in The Netherlands: an outbreak despite high levels of immunization with whole-cell vaccine. Emerg Infect Dis. 1997;3:175-178

53: Neppelenbroek SE, Melker HE de, Schellekens JFP, Conyn-van Spaendonck MAE: The incidence of pertussis in the Netherlands has remained high since an outbreak occurred in 1996. Euro Surveill. 1999;4:133-134

54: Melker HE de , Schellekens JFP, Neppelenbroek SE, Mooi FR, Rumke HC, Conyn-van Spaendonck MAE: Reemergence of pertussis in the highly vaccinated population of the Netherlands: observations on surveillance data. Emerg Infect Dis. 2000;6:348-357

55: Feer E: Ueber das Wesen und über die Infektionsverhältnisse des Keuchhustens. Med Klinik 1914;10: 837-841

56: Luttinger P: The epidemiology of pertussis. Am J Dis Child 1916;12:290-315

57: Schwenkenbecher A: Keuchhusten bei Erwachsenen. Med Klin 1921;17:1447-1448

58: Schlack H: Der Keuchhusten der Erwachsenen. Munch Med Wochenschr 1938;85: 1541-1544

59: Gordon JE, Hood RI: Whooping cough and its epidemiological anomalies. Am J Med Sci 1951;222: 333-361

60: Mooi FR, Loo IHM van, King AJ: Adaptation of *Bordetella pertussis* to vaccination: a cause for its reemergence? Emerg Infect Dis 2001;7(3 Suppl):526-528

61: Rohani P, Earn DJD, Grenfell B: Impact of immunisation on pertussis transmission in England and Wales. Lancet 2000;355:285-286

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

63: Gilberg S, Njamkepo E, Parent du Chatelet I, Partouche H, Gueirard P, Ghasarossian C, Schlumberger M, Guiso N: Evidence of *Bordetella pertussis* infection in adults presenting with persistent cough in a French area with very high whole-cell vaccine coverage. J Infect Dis 2002;186:415-418

64: Senzilet LD, Halperin SA, Spika JS, Alagaratnam M, Morris A, Smith B, Sentinel Health Unit Surveillance System Pertussis Working Group: Pertussis is a frequent cause of prolonged cough illness in adults and adolescents. Clin Infect Dis 2001;3212:1691-1697. Epub 2001 May 21

65: Long SS, Welkon CJ, Clark JL: Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis 1990;161:480-486

66: He Q, Viljanen MK, Nikkari S, Lyytikäinen R, Mertsola J: Outcomes of *Bordetella pertussis* infection in different age groups of an immunized population. J Infect Dis 1994;170: 873-877

67: WHO-Pertussis surveillance: WHO/V&B/01.19

68: Schellekens JFP, Melker HE de: Laboratoriumdiagnostiek van infecties met Bordetella spp. bij patiënt en populatie. Ned Tijdschr Med Microbiol 2000;8:112-116

69: Zee A van der, Agterberg C, Peeters M, Mooi F, Schellekens J: A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis 1996;174:89-96

70: Bordet J, Gengou O: Le diagnostic de la coqueluche fruste par la méthode de la fixation de lálexine. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 1 Orig 1911;58-68

71: Müller F-MC, Hoppe JE, Wirsing von König CH: Laboratory diagnosis of pertussis: state of the art in 1997. J Clin Microbiol 1997;35:2435-2443

72: Nagel J, de Graaf S, Schijf-Evers D: Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LPF antibody levels. Dev Biol Stand 1985;61:325-330

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

74: Bergquist SO, Bernander S, Dahnsjo H, Sundelof B: Erythromycin in the treatment of pertussis: a study of bacteriologic and clinical effects. Pediatr Infect Dis J 1987;6:458-461.Erratum in: Pediatr Infect Dis J 1987;6:1035

75: Aoyama T, Sunakawa K, Iwata S, Takeuchi Y, Fujii R: Efficacy of short-term treatment of pertussis with clarithromycin and azithromycin. J Pediatr 1996;129:761-764

76: Lebel MH, Mehra S: Efficacy and safety of clarithromycin versus erythromycin for the treatment of pertussis: a prospective, randomized, single blind trial. Pediatr Infect Dis J 2001;20:1149-115

77: Langley JM, Halperin SA, Boucher FD, Smith B; Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC): Azithromycin is as effective as and better tolerated than erythromycin estolate for the treatment of pertussis. Pediatrics 2004;114:e96-101

78: Halperin SA, Bortolussi R, Langley JM, Miller B, Eastwood BJ: Seven days of erythromycin estolate is as effective as fourteen days for the treatment of *Bordetella pertussis* infections. Pediatrics 1997;100:65-71

79: Bace A, Zrnic T, Begovac J, Kuzmanovic N, Culig J: Short-term treatment of pertussis with azithromycin in infants and young children. Eur J Clin Microbiol Infect Dis 1999;18:296-298

80: [No authors listed] Erythromycin-resistant *Bordetella pertussis*--Yuma County, Arizona, May-October 1994. MMWR Morb Mortal Wkly Rep 1994;43:807-810

81: Lewis K, Saubolle MA, Tenover FC, Rudinsky MF, Barbour SD, Cherry JD: Pertussis caused by an erythromycin-resistant strain of *Bordetella pertussis*. Pediatr Infect Dis J 1995;14:388-391

82: Hauben M, Amsden GW: The association of erythromycin and infantile hypertrophic pyloric stenosis: causal or coincidental? Drug Saf 2002;25:929-942

83: Honein MA, Paulozzi LJ, Himelright IM, Lee B, Cragan JD, Patterson L, Correa A, Hall S, Erickson JD: Infantile hypertrophic pyloric stenosis after pertussis prophylaxis with erythromcyin: a case review and cohort study. Lancet. 1999;354:2101-2105. Erratum in: Lancet 2000;355:758

84: Hoppe JE, Halm U, Hagedorn HJ, Kraminer-Hagedorn A: Comparison of erythromycin ethylsuccinate and co-trimoxazole for treatment of pertussis. Infection 1989;17:227-231. Erratum in: Infection 1989;17:330

85: Preston NW, Kerr JR: Current pharmacotherapy of pertussis. Expert Opin Pharmacother 2001;2: 1275-1282

86: Dekking F: Behandeling van pertussis met hyperimmune gammaglobuline. Ned Tijdschr Geneeskd 1972;116:1023

87: 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

88: Casey PA: Altitude treatment for whooping cough. BMJ 1991;302:1212

89: Hall D: Altitude treatment for whooping cough. BMJ. 1991;303:58

90: Kostadima E, Tsiodras S, Alexopoulos EI, Kaditis AG, Mavrou I, Georgatou N, Papamichalopoulos A: Clarithromycin reduces the severity of bronchial hyperresponsiveness in patients with asthma. Eur Respir J 2004;23:714-717

91: LCI-protocol Pertussis-kinkhoest. Den Haag, LCI 1999/2000

92: Jefferson T, Rudin M, DiPietrantonj C: Systematic review of the effects of pertussis vaccines in children. Vaccine 2003;21:2003-2014

93: Bisgard KM, Pascual FB, Ehresmann KR, Miller CA, Cianfrini C, Jennings CE, Rebmann CA, Gabel J, Schauer SL, Lett SM: Infant pertussis: who was the source? Pediatr Infect Dis J 2004;23:985-989

94: Kostalova B (red.). Kinkhoest totaal 2000 - 2004. In: Volksgezondheid Toekomst Verkenning, Nationale Atlas Volksgezondheid. Bilthoven: RIVM, http://www.zorgatlas.nl Gezondheid en ziekte\Ziekten en aandoeningen\Infectieziekten, 15 september 2004, http://www.rivm.nl/vtv/data/atlas/zktn_infz_a007.htm

Chapter 2

Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*

H.E. de Melker [1], F.G.A. Versteegh [2], M.A.E. Conyn-van Spaendonck [1],

L.H. Elvers [3], G.A.M. Berbers [4], A. van der Zee [5], J.F.P. Schellekens [3]

1: Department of Infectious Diseases Epidemiology, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

2: Department of Pediatrics, 'Groene Hart' Hospital, Gouda, The Netherlands

3: Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

4: Laboratory for Clinical Vaccine Research, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

5: Laboratory of Medical Microbiology, St. Elisabeth Hospital, Tilburg, The Netherlands

Abstract

Laboratory confirmation of pertussis by culture, PCR, or detection of antibody increase in paired sera is hampered by low sensitivity in the later stages of the disease. Therefore, we investigated whether and at which level concentrations of immunoglobulin G (IgG) antibodies against pertussis toxin (PT), IgG-PT, in a single serum sample, are indicative of active or recent pertussis. IgG-PT, as measured by enzyme-linked immunosorbent assay in units per milliliter, was analyzed in 7,756 sera collected in a population-based study in The Netherlands, in the sera of 3,491 patients with at least a fourfold increase of IgG-PT, in paired sera of 89 patients with positive cultures and/or PCR results and in the sera of 57 patients with clinically documented pertussis with a median follow-up of 1.4 years. We conclude that, independent of age, IgG-PT levels of at least 100 U/ml are diagnostic of recent or active infection with *Bordetella pertussis*. Such levels are present in less than 1% of the population and are reached in most pertussis patients within 4 weeks after disease onset and persist only temporarily.

Introduction

Whooping cough is a highly contagious bacterial infection of the respiratory tract, caused by *Bordetella pertussis*. It is most severe in unvaccinated infants. Evidence is increasing that *B. pertussis* infections occur more frequently in older children and adults in vaccinated populations than has been commonly recognized (1, 4, 6, 8, 18, 23, 27). These individuals may play an important role in the transmission to infants too young to be vaccinated (4, 7, 20, 26). Adequate laboratory diagnosis is important for the control and prevention of pertussis. In The Netherlands, the case definition for notification of pertussis includes defined typical clinical symptoms and laboratory confirmation. Laboratory confirmation is defined as either a positive culture or a positive PCR for *B. pertussis* or *B. parapertussis,* or positive two-point serology, i.e., a significant increase (at least fourfold) of antibodies against (antigens of) *B. pertussis*. This case definition for notification is highly specific but it results in low sensitivity, especially when laboratory diagnosis is initiated at a late stage of the disease. Other countries also report that pertussis diagnosis is hampered by low sensitivity (17, 21, 28, 31, 32). Culture of *B. pertussis* is laborious and insensitive; the ability to isolate *B. pertussis* by culture decreases progressively during the disease (13, 14). The sensitivity of PCR is superior to that of culture; however, this sensitivity, like that of culture, rapidly decreases by the time the paroxysmal phase has developed and with increasing age (16, 35). In The Netherlands, confirmation of suspected pertussis is attempted often by serology. However, in our serodiagnostic practice, for more than 50% of the suspected cases, only one serum sample is submitted or else high titers are found in paired sera without a significant increase. Similar problems have also been

reported by others (10, 24, 29).

Because pertussis toxin (PT) is expressed only by *B. pertussis* and cross-reacting antigens have not been described (15, 24) and because immunoglobulin G (IgG) responses occur in most patients with *B. pertussis* infection, we investigated whether and at which level, titers of IgG antibodies against PT (IgG-PT) in a single serum sample are indicative for active or recent pertussis.

We analyzed IgG-PT in the sera of a large cross-section of the population (*n* = 7,756), in the paired sera of patients of all ages in whom clinical suspicion of pertussis was confirmed by at least a fourfold increase of IgG-PT (*n* = 3,491) and in the paired sera of patients in whom pertussis had been confirmed by culture of *B. pertussis* or by positive pertussis PCR (*n* = 89). The course of IgG-PT after natural infection, i.e., the duration of high levels, was assessed in long-term follow-up sera of 57 patients after pertussis had been clinically documented.

Materials and Methods

Collection of sera and data from the general population and patients. (i) Cross-section of the general population (*n* = 7,756). The study design and data collection have been published elsewhere (22). Briefly, eight municipalities with probabilities proportional to their population sizes were sampled within each of five geographical Dutch regions with similar population sizes. An age-stratified sample (classes 0, 1 to 4, 5 to 9,..., 75 to 79 years) of 380 individuals was randomly selected from each municipality. These individuals were requested to give a blood sample and to fill out a questionnaire in which the participants were asked whether they had had a period with coughing attacks that had lasted for more than 2 weeks. They were also asked whether a physician had diagnosed pertussis, either during the past year or for more than 1 year previously. No information was available as to whether the physician had diagnosed pertussis by symptomatology, serology, culture, or PCR. The participation rate was 55%. Sufficient serum for pertussis serology was available from 7,756 of the 8,359 participants. Sera were collected in 1995 and 1996 and stored at -70°C until use.

(ii) Patients with serologically confirmed pertussis. Until 1997, the National Institute of Public Health and the Environment was the only laboratory in The Netherlands that performed pertussis serology examinations for patients with a suspected pertussis infection. Routinely, the submitted sera were assayed for both IgG-PT and IgA against *B. pertussis*. In all cases, if the date of onset of symptoms and/or date of sampling of serum was missing upon submission of serum, a standard questionnaire was sent to collect the data. From January 1989 onwards, all data and results were registered in an electronic database.

For the purpose of this study, patient data and serologic results were obtained in the period from 1989 to 1996 from 3,491 patients in whom the clinical suspicion of pertussis had been confirmed by the detection, in paired sera, of a ≥4-fold increase of IgG-PT to ≥20 U/ml.

Likewise, data were analyzed for 15,319 patients whose first submitted serum sample contained ≥100 U of IgG-PT per ml without (in the case of paired sera) a fourfold IgG-PT increase.

(iii) Patients with typically symptomatic infection with *B. pertussis* and their longitudinal sera. During the period from 1989 to 1998, we obtained follow-up serum samples from 57 patients with a clinical diagnosis of typical pertussis (paroxysmal cough lasting more than 2 weeks) so that we could study the longitudinal course of IgG-PT after infection. Twenty-three patients showed at least a fourfold increase in IgG-PT in paired sera and 34 patients had an IgG-PT level in a first serum sample of at least 75 U/ml. The IgG-PT level was at least 100 U/ml for 31 of these 34 patients. The follow-up period varied from 6.5 months to 6.7 years after the acute phase of infection (mean, 1.8 years; median, 1.4 years). The number of serum samples collected in the follow-up periods varied from two to seven (mean and median of three). All patients were from a single pediatric practice. The patients were treated with macrolides for their pertussis. Only those patients participated who continued to be treated by the pediatrician after the episode of pertussis because of other medical conditions (mostly allergic conditions and/or asthma) or who consulted the pediatrician again at a later stage because of new medical problems. In addition, sera from parents with clinical pertussis were selected. The median age of the patients in which the longitudinal course of IgG-PT was studied was 3.5 years (range, 0 to 35 years). A total of 10 patients were less than 6 months old; 7 patients were 6 to 11 months old; 19 patients were between 1 and 4 years old, 16 patients were between 5 and 9 years old; 2 patients were 11 or 12 years old; and 3 patients were 30 to 35 years old. Thirty-nine of the patients were vaccinated, six patients were unvaccinated and for twelve patients the vaccination status was unknown. In all cases the follow-up sera used for the study were "left over" from samples obtained for some other diagnostic procedure. Informed consent for the study was obtained from the patients or their parents.

(iv) Patients with PCR and/or culture-proven pertussis. In the period from 1993 to 1998, the diagnosis of pertussis for 89 patients had been confirmed by culture of *B. pertussis* and/or a positive pertussis-specific PCR, while paired sera from these patients had also been submitted for serology. Of the 89 patients, 58 had participated in a clinical study to assess the sensitivity of the pertussis or parapertussis PCR in comparison with culture and serology (35). For each of the remaining patients, a pertussis PCR test was performed in the regional public health laboratory in Tilburg, The Netherlands. In all cases, the first serum of the pair was obtained on the same day that material for culture and/or PCR had been obtained: the second sample was obtained 2 to 4 weeks later. Of the 89 patients, 37.1% were 0 to 5 months old, 7.9% were 6 to 11 months old, 21.3% were 1 to 4 years old, 25.2% were 5 to 9 years old, 2.2% were 10 to 14 years old and 5.6% were ≥15 years old.

In-house IgG-PT ELISA. The patient sera had been submitted immediately after sampling and were assayed in the routine setting of the serology laboratory of our institute within 4 days after receipt. The population sera, which had been collected in 1995 and 1996, were assayed

in 1997 and 1998 in the same routine setting at a rate of approximately 200 sera per week. The IgG-PT was measured by enzyme-linked immunosorbent assay (ELISA) as previously described (35). In short, the procedure was as follows. Purified PT (National Institute of Public Health and the Environment) was used to coat 96-well ELISA plates after precoating with fetuin (50 mg/liter in phosphate-buffered saline). Peroxidase-labeled rabbit anti-human IgG was used as a conjugate and 3,3',5,5'-tetrameth-ylbenzidine (TMB) was used as the substrate. Negative, low-positive and medium-positive control sera with defined IgG-PT contents were run on each plate. The sera were tested in duplicate in 1:100 and 1:400 dilutions against serial dilutions of a positive reference serum with a range of 1.6 to 100 "local" U/ml. The optical density (OD) of the 1:100 dilution was used to calculate the IgG-PT concentration. When the OD of the 1:100 dilution of a serum was above the range that constituted the steep part of the dose-response curve, the OD of the 1:400 dilution was used.

Due to the use of only two dilutions for the sera, the IgG-PT assay has an upper limit (500 U/ml) above which the values are not further differentiated. The lower detection limit of the assay is 5 U/ml.

Results are expressed in "local" units per milliliter. The reference serum was also calibrated against the FDA preparation lot 3 (Food and Drug Administration Laboratory of Pertussis, Rockville, Md.). The formula for conversion of local to FDA units per milliliter appeared to be as follows: local U/ml x 1.25 = FDA U/ml within this assay (9).

Data analysis. For the cross-sectional population-based study, frequencies of IgG-PT levels from <5 U/ml (lower detection limit) to ≥500 U/ml (upper differentiation limit) within each municipality were weighted by the proportion of the age group in the population. To produce national estimates for percentiles 1, 2.5, 10, 25, 50, 75, 97.5 and 99, these weighted and age-specific frequencies were averaged over the 40 municipalities (5). The following age groups were separately analyzed: 0 to 5 months, 6 to 11 months, 12 to 17 months, 18 to 23 months, 2 years, 3 years, 4 years, 5 to 9 years, 10 to 14 years and ≥15 years.

The proportions with IgG-PT levels of <5U/ml, 5 to 9 U/ml, 10 to 49 U/ml, 50 to 99 U/ml, 100 to 499 U/ml and ≥500 U/ml were also assessed. The proportion of these groups that reported a pertussis diagnosis or a period with coughing attacks during the past year was calculated. The proportion of those who had a pertussis diagnosis and/or coughing attacks more than one year ago and the proportion without any pertussis diagnosis or coughing attacks were also calculated.

Likewise, for the patients with serologically proven pertussis (i.e., a ≥4-fold increase of IgG-PT in paired sera), the total and (similar) age-specific percentiles 1, 2.5, 10, 25, 50, 75, 97.5 and 99 for IgG-PT levels were calculated for the second sera of the serum pairs. The median duration of disease at the time of initiation of laboratory diagnosis, i.e., the time of sampling of first sera, was assessed for these patients in the following age groups: 0 to 5 months, 6 to 11 months, 1 year, 2 years, 3 years, 4 years, 5 to 9 years, 10 to 14 years and ≥15 years.

The median duration of the disease was also assessed for the same age groups for the selection of patients from the serological database with ≥100 U/ml at the time of the first serum sampling without a fourfold IgG-PT increase.

The Wilcoxon signed ranked test was used to test differences between age groups in the IgG-PT distributions in the patients with positive cultures and/or PCR with a *P* value of 0.05, which was considered statistically significant.

To study the longitudinal course of IgG-PT levels after pertussis infection for patients in the follow-up study, the association between the IgG-PT level and time in ^{2}log days after the first day of illness (and the effect of age) was analyzed with a mixed linear model (PROC MIXED in SAS version 6.12) (30). A ^{2}log transformation of both the IgG-PT level in units per milliliter and of time in days since the first day of illness was performed. This way, linear regression yielded the best fit.

For those patients with follow-up data at 0 to 5 months, 6 to 11 months, 12 to 23 months, 2 to 3 years and 4 to 7 years after onset of the disease, the proportions with IgG-PT levels of <20, 20 to 49, 50 to 99 and ≥100 U/ml were calculated. If multiple serum samples were available for one patient within one follow-up period, the serum sample with the highest IgG-PT level was used.

Results

The IgG-PT distribution in the population compared to the IgG-PT distribution in second sera of serum pairs of patients with serologically confirmed pertussis: choice of cut-offs to be validated. The IgG-PT levels in individuals of the population-based study were low in comparison to those in second sera (i.e., reconvalescence sera) of patients with serologically confirmed pertussis; these distributions showed little overlap (Fig. 1). In the population-based study, the median IgG-PT was 6 U/ml and for percentiles 97.5 and 99 the values were 69 and 97 U/ml, respectively. In the second sera of patients with serologically confirmed pertussis, the median IgG-PT was ≥500 U/ml; the 10th percentile was 66 U/ml (Fig. 1).

In the population-based study, the median IgG-PT level was <5 U/ml in the age group <10 years old, rose to 5 U/ml for 10 to 14-year-olds and was 7 U/ml for patients 15 years old or older (Fig. 1). For those 0 to 5 months old, 6 to 11 months old and 18 to 23 months old, the percentile 97.5 value was less than 20 U/ml, while in the other age groups the values given by percentile 97.5 ranged from 26 U/ml (4-year-olds) to 98 U/ml (10-to 14-year-olds). Further differentiation in 5-year age classes from 15 to 19 years to 75 to 79 years showed a stable IgG-PT distribution for those who were ≥15 years old (data not shown separately). With the exception of the 10-to 14-year-olds, the age-specific percentile 99 value was below 100 U/ml. The percentages with undetectable IgG-PT (i.e., <5 U/ml) decreased from 85.5% for infants aged 6 to 11 months to 67.4% for infants 12 to 17 months and increased again to 79.4% at the

age of 4 years (Fig. 1). The proportion with undetectable IgG-PT decreased to 31.7% in those ≥15 years of age. From this age onwards, this percentage remained stable (data not shown separately).

The median and the 10th percentile of the distributions of IgG-PT in the second sera of patients with serologically proven pertussis showed little variation in the different age groups; the median IgG-PT values ranged from 316 to ≥500 U/ml and the 10th percentile values ranged from 40 to 87 U/ml (Fig. 1). The percentage of patients with an IgG-PT level of ≥500 U/ml ranged from 40.7 to 57.0% within the various age groups (Fig. 1). Thus, the IgG-PT distributions in the population differed for the various age categories but the IgG-PT immune responses in pertussis patients did not. Therefore, for further assessment of specificity and sensitivity of certain IgG-PT levels for recent and/or active infection with *B. pertussis*, we chose cutoffs of IgG-PT levels which were age independent, i.e., which were based on the comparison of the IgG-PT distribution in the total population and the IgG-PT distribution in second sera of the total group of serologically confirmed pertussis patients. Cutoffs of 50 and 100 U/ml were chosen because IgG-PT levels of ≥50 and ≥100 U/ml were detected in, respectively, 92.7 and 81.0% of patients (second sera) with serologically confirmed pertussis. Such levels were rare, i.e., respectively, 3.6 and 0.8% in the general population. That is to say, they gave specificities of such values as 96.4 and 99.2%, respectively. The specificity of the proposed cutoff of ≥100 U/ml was maximally 100% for the ages of 0 to 5 months, 12 to 17 months, 18 to 23 months and 2 years; minimally 97.9% for 10-to 14-year-olds; and for the cutoff value, ≥50 U/ml, maximally 100% for the ages of 0 to 5 months and 18 to 23 months and minimally 96.2% for 10-to 14-year-olds and ≥15-year-olds.
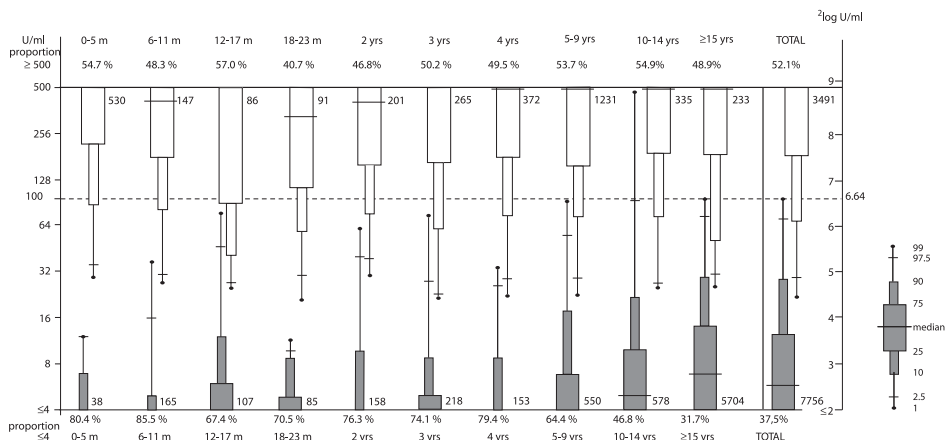

Fig 1: IgG-PT distribution in the population-based study and in pertussis patients (second sera) with at least a fourfold increase in IgG-PT level in paired sera. Results for the population-based study are given in shaded bars and results for second sera of pertussis patients are given in open bars; the total numbers of individuals/patients are given beside the bars.

Validation of the proposed IgG-PT cutoffs as markers for recent or active infection with *B. pertussis*. (i) Relationship between high IgG-PT levels in the population and recall of pertussis diagnosis and/or paroxysmal coughing. In the population-based study, while 9.6% of those with an IgG-PT level of <5 U/ml reported coughing attacks or pertussis during the last year, this percentage was statistically significantly greater, being 19.9% for those with an IgG-PT level between 50 and 99 U/ml and 25.5% for those with an IgG-PT level of ≥100 U/ml (Table 1).

The percentage of individuals who reported that pertussis was diagnosed or who reported to have had coughing attacks more than 1 year ago showed a smaller increase with increasing IgG-PT. The differences in the percentage were not statistically significant (Table 1).

TABLE 1. Percentages of individuals with a history of pertussis or coughing attacks during the last year, of individuals with pertussis or coughing attacks more than 1 year ago, and of individuals without pertussis or coughing attacks according to IgG antibody levels in the population-based study[a]

Occurrence pertussis or coughing attack status	n	% Individuals with IgG concn of:									
		<5 U/ml		5–9 U/ml		10–49 U/ml		50–99 U/ml		≥100 U/ml	
		%	95% CI[a]	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Yes, during last year[b]	886	9.6	8.0–11.3	11.6	9.4–13.8	11.3	9.7–12.9	19.9	13.0–26.9	25.5	12.2–38.8
Yes, more than 1 year ago[c]	463	5.5	4.3–6.7	6.1	4.6–7.6	6.7	5.2–8.5	7.3	3.5–11.0	14.1	2.7–25.5
No[d]	6,407	84.9	82.8–87.0	82.3	79.9–84.6	81.8	80.1–83.5	72.8	65.2–80.5	60.4	45.3–75.5
Total	7,756	100		100		100		100		100	

[a] CI, confidence interval.
[b] Participants who reported that a physician diagnosed pertussis during the last year or participants who reported coughing attacks that had lasted for more than 2 weeks during the last year. Among the 886 individuals, 15 individuals reported pertussis, and 12 of these 15 individuals reported coughing attacks.
[c] Participants who reported that a physician diagnosed pertussis more than 1 year ago or participants who reported coughing attacks that had lasted for more than 2 weeks more than 1 year ago. No pertussis was diagnosed and no coughing attacks were reported during the last year. Among the 473 individuals, 126 individuals reported pertussis, and 45 of these individuals reported coughing attacks.
[d] Participants who did not report pertussis or coughing attacks during the last year or more than one year ago.

(ii) Longitudinal course of IgG-PT levels after infection. In each of the 57 patients with clinical pertussis for whom there were follow-up serum samples, the IgG-PT decreased with time after the onset of the disease (Fig. 2). In the mixed linear model with the IgG-PT in ^{2}log U/ml and time from the onset of the disease in ^{2}log days, the intercept amounted to 14.61 and the slope was -1.128. Thus, this model predicts that the mean time of persistence of an IgG-PT level of ≥100 U/ml amounts to 134 days (4.4 months) and that after 365 days a level of 32 U/ml is reached. Although IgG-PT levels of ≥500 U/ml were not further differentiated, the slopes did not change statistically significantly assuming that the levels of ≥500 U/ml were 1,000 U/ml or restricting the analysis to those samples with IgG-PT levels of ≥500 U/ml. No effect of age or vaccination status was shown in the mixed linear model. The data points for each individual patient were connected linearly. The resulting lines show that the IgG-PT level was below 100 U/ml within 1 year for 47 of the 57 patients. For 7 of the remaining 10 patients, the IgG-PT levels decreased below 100 U/ml during the subsequent follow-up period, which ranged from 1.4 to 4 years. For two of the remaining three patients, the follow-up period was less than 1 year. For these two patients, the IgG-PT levels amounted to 160 and 304 U/ml after 0.84 and 0.92 year of follow-up, respectively. The remaining patient had an IgG-PT level of 252 U/ml at 1.1 years after the first day of illness.
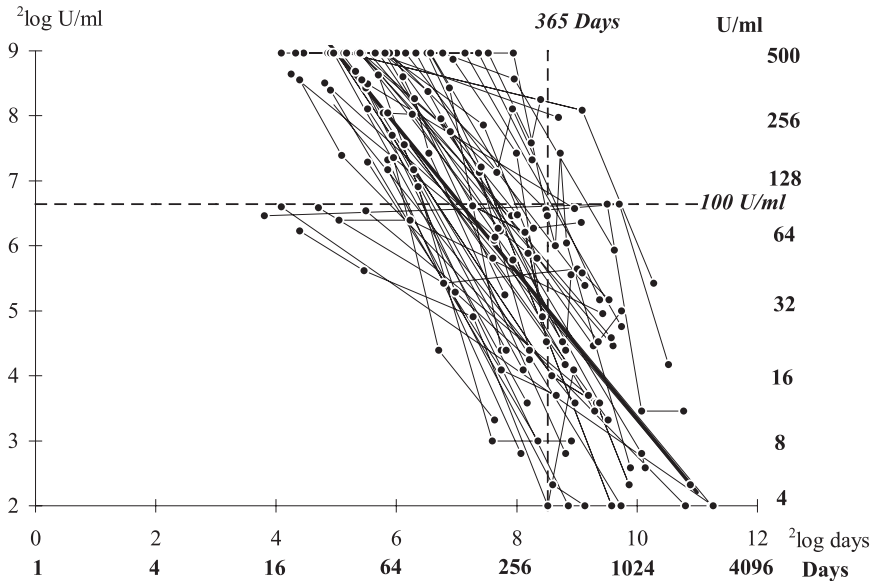
Fig 2: IgG-PT levels (in ^{2}log U/ml) versus time elapsed (in ^{2}log days) since date of onset for 57 patients with clinical pertussis.

As shown in Table 2, the highest IgG-PT level detected between 0 and 5 months after onset of the disease was ≥100 U/ml in 90% of the patients, while the highest IgG-PT level detected in the remaining patients was between 50 and 100 U/ml. At 6 to 11 months after the onset of the disease, the level had declined to <50 U/ml in 40% of the patients. A decrease to <50 U/ml had occurred in 72% after 12 to 23 months, in 86% after 2 to 3 years and in 100% after 4 to 7 years (Table 2).

TABLE 2. IgG-PT levels in patients with clinical pertussis
versus time elapsed since disease onset

IgG-PT level (U/ml)	% of patients with indicated IgG-PT level at time of serum collection after onset of disease[a]				
	0–5 mo (n = 57)	6–11 mo (n = 30)	12–23 mo (n = 25)	2–3 yr (n = 14)	4–7 yr (n = 8)
≥100	90	20	16	7	0
50–99	11	40	12	7	0
20–49	0	13	28	36	13
<20	0	27	44	50	88

[a] *n*, number of patients.

(iii) Time between onset of the disease and development of high IgG-PT levels. Sera from patients for all age groups with an IgG-PT level of ≥100 U/ml in the first serum sample without a fourfold increase in IgG-PT level had been sampled at a later stage of the disease (median 30 days) than the first sera of patients showing a fourfold increase in IgG-PT level (median 17 days) (Table 3). The first samples from patients younger than 3 years of age were collected a few days earlier than the samples from older age groups, particularly

TABLE 3. Time between onset of the disease and first blood sampling for patients with at least a fourfold IgG-PT increase and
for patients with at least 100 U/ml in the first serum sample without a fourfold IgG-PT increase

Age	Patients with at least a fourfold IgG-PT increase				Patients with at least 100 U/ml in first serum sample without a fourfold IgG-PT increase			
	No. of patients	Median (days)	Percentiles 2.5–97.5	Mean (days)	No. of patients	Median (days)	Percentiles 2.5–97.5	Mean (days)
0–5 mo	517	14	2–53	17.0	497	30	4–108	36.8
6–11 mo	144	14	2–61	16.8	305	29	2–105	35.4
1 yr	174	15	2–62	19.2	426	28	4–122	36.3
2 yr	195	15	4–88	19.6	763	30	6–109	38.1
3 yr	262	18	3–60	19.7	966	30	7–119	38.5
4 yr	362	21	5–55	21.9	1,499	31	7–112	39.0
5–9 yr	1,191	18	3–58	20.6	5,655	31	7–103	37.6
10–14 yr	327	18	4–61	21.3	2,180	31	7–111	40.0
≥15 yr	222	16	3–67	19.3	3,028	30	6–108	37.2
Total	3,394[a]	17	3–59	19.8	15,319	30	6–109	38.0

[a] $n < 3,491$ due to missing values for time of disease onset.

for patients with at least a fourfold increase in IgG-PT.

The results given in Table 3 also show the number of patients with an IgG-PT level of ≥100 U/ml in a first serum that in our serodiagnostic database of 1989 to 1996 is considerably larger (4.5-fold) than the number of patients with at least a 4-fold increase in IgG-PT. This discrepancy increases with age from 1-fold for the 0-to 5-month-old ages to 13.6-fold for those of ≥15 years.
(iv) Sensitivity of the proposed cutoffs for IgG-PT in PCR and/or culture-confirmed pertussis cases. Among the 89 patients for whom pertussis was confirmed by a positive culture and/or a PCR both in the IgG-PT distributions in the first and second serum samples, no statistically significant differences were found between age groups. The distributions of IgG-PT in the first and second sera of the serum pairs of these patients are shown in Fig. 3. In 3 of the 89 patients, IgG-PT was undetectable (<5 U/ml) in both the first and the second serum samples. In two other patients the IgG-PT rose from <5to7 and 8 U/ml. In 69 patients a ≥4-fold increase of IgG-PT was detected, in 11 patients the IgG-PT in the first serum sample was ≥100 U/ml and no fourfold rise was detected and in 4 patients the rise in IgG-PT was <4-fold and the IgG-PT level in the first serum sample was <100 U/ml. Thus, if detection of a ≥4-fold increase of IgG-PT in paired sera and detection of ≥100 U of IgG-PT per ml in a single serum sample are used as the criteria for the serodiagnosis of active or recent infection with *B. pertussis*, the sensitivity of IgG-PT serology in this "gold standard" group of patients is enhanced from 77.5% (69 of 89) to 89.9% (80 of 89). Overall, the sensitivity of IgG-PT levels of ≥50 and ≥100 U/ml amounted to 88.8 and 76.4%, respectively; i.e., 79 and 68 of the 89 patients had IgG-PT levels of ≥50 and ≥100 U/ml in the first and/or second serum samples respectively.

Discussion

Our results show that an IgG-PT level of at least 100 U/ml is a specific tool in laboratory confirmation of patients with a suspected pertussis infection in The Netherlands. The levels

first sera
N=89

second sera
N=89

100 U/ml

Igg-PT U/ml
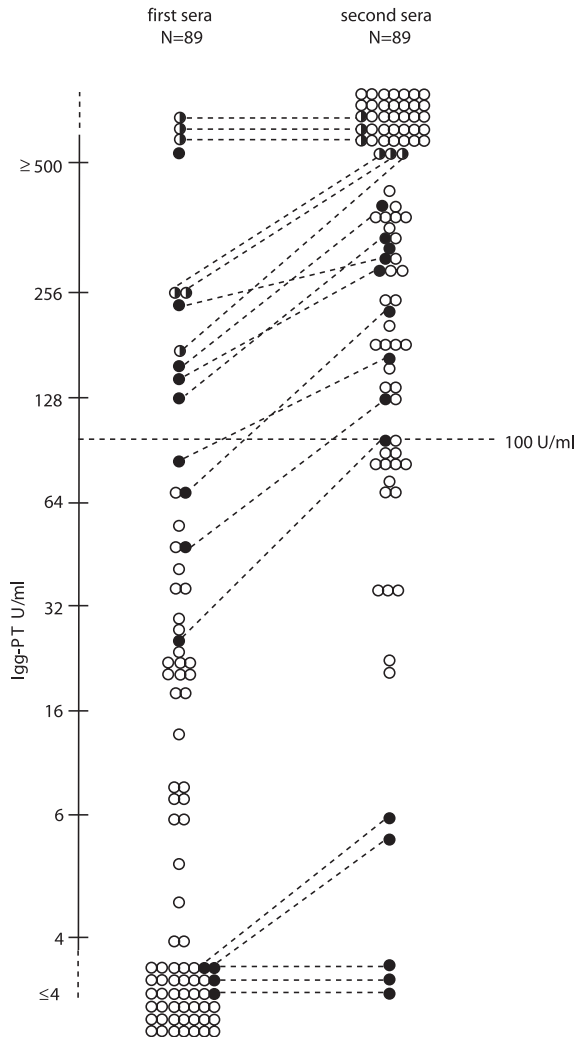
Fig 3: IgG-PT distribution in the first and second serum samples of patients with a positive culture and/or PCR for *B. pertussis*. White circles indicate sera with at least a fourfold increase in IgG-PT to a level of at least 20 U/ml. Black circles indicate sera without a fourfold increase in IgG-PT; pairs of sera are connected with a dotted line. Half-black, half-white circles indicate sera in which the degree of increase of IgG-PT could not be determined due to the upper limit of differentiation of the IgG-PT assay of ≥500 U/ml; pairs of sera are connected with a dotted line.

of IgG-PT in the Dutch population are lower than and overlap only slightly, IgG-PT levels that are reached in patients with clinical symptoms of pertussis and a significant immune response to *B. pertussis*. IgG-PT levels of at least 100 U/ml were observed in <1% of the overall general population, varying between maximally 2.5% for 10-to 14-year-olds and <0.5%

for those 2 years of age or younger. Furthermore, such levels were present in the second serum sample of 80% of those patients who had at least a fourfold increase in IgG-PT.

Our longitudinal study of pertussis patients shows that the levels decreased within less than 1 year to a level below 100 U/ ml after natural infection with *B. pertussis* for almost all patients who had had high IgG-PT levels. The regression model predicts that a level of 100 U/ml is reached in 4.5 months and a level of <40 U/ml is reached within 1 year after onset of the disease. For seven of eight patients with a longer follow-up time, the IgG-PT levels were below 20 U/ml by 4 to 7 years after the disease onset. Thus, IgG-PT levels of at least 100 U/ml for patients with suspected whooping cough are indicative of recent or active infection with *B. pertussis*.

In our population-based study, the percentage of individuals who reported pertussis or coughing attacks in the last year increased from 10% for those with IgG-PT levels of <5 U/ml to 26% for those with levels of at least 100 U/ml. This finding offers further support for our conclusion that a high IgG-PT level is indicative of recent or active infection with *B. pertussis*. Ten percent of the individuals in the population study with an IgG-PT level of <5 U/ml reported pertussis or coughing attacks in the last year. On the one hand, this may be explained by other respiratory tract infections that cause "pertussis-like" symptoms (36), rapid decline of a previously high IgG-PT level, or absence of an IgG-PT response after *B. pertussis* infection. It is also possible that not all subjects answered the question properly. On the other hand, the large percentage (60%) of individuals with high antibody titers who did not report pertussis or long-lasting coughing attacks might be due to very mild, atypical or even asymptomatic infection with *B. pertussis*. In a household exposure study, a *B. pertussis* infection was shown to exist in 46% of the exposed subjects who remained well (7). In another study, only 26% of the adults with laboratory evidence of a *B. pertussis* infection reported recent symptoms compatible with pertussis (31).

Although IgG-PT is induced after whole-cell vaccination only in children aged 12 to 17 months and thus after the fourth dose given at the age of 11 months, a temporary and small increase was observed. These results are consistent with observations in a vaccine trial showing very low levels of IgG-PT after the first to third vaccinations and a small increase just after the fourth vaccination as Nagel and others have observed (19, 25). Thus, it is very unlikely that high levels of IgG-PT are induced by previous vaccination with Dutch whole-cell vaccine. However, other vaccines might induce higher IgG-PT levels since the response to PT varies between different whole-cell vaccines and acellular vaccines (2, 12, 33, 34). However, even when a level of at least 100 U/ml is reached, it is likely to decline shortly afterwards (12, 33, 34). Giuliano et al. (12) reported that mean titers were close to the limit of detection 15 months after the primary immunization with acellular vaccine. High IgG-PT levels must be interpreted more cautiously in children recently vaccinated with a vaccine known to induce relatively high levels of such antibodies.

In addition to specificity, both sensitivity and positive predictive value are important as diagnostic tools. Using paired sera of patients with positive PCRs and/or positive cultures (gold standard group), a sensitivity of IgG-PT of at least 100 U/ml was 76%. At least a fourfold increase was found in most of the remaining patients in whose paired sera the IgG-PT level remained lower than 100 U/ml. One might speculate that a level of at least 100 U/ml may have been reached at a later point. Only 6% of all patients had very low IgG-PT levels in both sera without significant dynamics. With the exception of those aged 10 to 14 years, a level of IgG-PT of at least 100 U/ml exceeded the 99th percentile in the general population. Furthermore, as described above, it was likely that individuals in the general population with IgG-PT levels above this value had had a recent or active *B. pertussis* infection. Based on this 99th percentile, the positive predictive values will still amount to 90 and 80%, assuming the proportions of true pertussis patients to be 9 and 4%, respectively, among those who submitted serum samples [i.e., 9/(9 + 1) and 4/(4 + 1)]. However, depending on the clinical presentation and the epidemiological situation, the a priori chance of true positivity in most cases will be higher. Using a more conservative estimate, i.e., percentile 97.5 in the general population, the positive predictive value will not be below 80%, assuming a percentage of true positives of 10%.

Even an IgG-PT level of at least 50 U/ml has some predictive value, since it amounts to 70%, assuming a percentage of true positives of 10%. This suggests that the diagnosis of pertussis is likely among patients with clinical symptoms of pertussis with such IgG-PT levels. However, we interpret such a result to be indicative of but not definite proof of, a recent *B. pertussis* infection and we advise submission of a second serum sample. If no further change of IgG-PT level has occurred as evidenced by a second serum sample, we conclude that "recent or active infection with *B. pertussis* is possible."

Serological data obtained at our laboratory show that using our cutoff value for the IgG-PT level of at least 100 U/ml would increase the number of patients with serologically proven pertussis by more than fourfold. The increase is smallest for infants and greatest for adults, which is probably related to a longer delay in consulting a physician and/or initiating laboratory testing in older children and adults. This is supported by the similar median time (28 to 30 days) between the first blood sampling and the onset of symptoms for the various age groups for those with an IgG-PT level of at least 100 U/ml. For patients with at least a fourfold increase in IgG-PT the median time (17 days) between the first blood sampling and the first symptoms is about 2 weeks shorter than that for those without a fourfold increase and IgG-PT levels above 100 U/ml. The most useful method for pertussis diagnosis depends on the time of initiation. PCRs and cultures are most useful early in the disease. However, if they are negative, the diagnosis is indeterminate and serology tests should be initiated. Late in the disease, PCRs and cultures are fairly insensitive (with an exception for infants less than 1 year old) and serology is then the method of choice (16, 21, 35).

To diagnose pertussis, other investigators have also used single serum samples from a control

group for defining a cutoff but most studies were limited to a specific study setting and were not meant for routine diagnosis (1, 6, 20, 21, 23, 27, 28, 32). Our control group consisted of a large number of participants from a population-based study so the representativeness is probably better guaranteed.

Cattaneo et al. point out that it is unlikely that a single serology value can be used to define infected persons in a broad age range because age, geographic area, prevalence of infection and history of vaccination all have to be taken into account (3). Yet an IgG-PT level of at least 100 U/ml in a single serum sample might be a specific diagnostic tool for pertussis in other countries too. After all, it is likely that such high IgG-PT levels will not or will be reached only temporarily no matter which vaccine is used. After the initial increase, the IgG-PT level decreases again after *B. pertussis* infection, high predictive values are calculated under different assumptions about prevalence of infection and, finally, a large proportion of individuals with a *B. pertussis* infection show high IgG-PT levels later in the course of the disease. Thus, we believe that high IgG-PT levels could provide a useful laboratory tool for the diagnosis of pertussis in both the individual patient and in epidemiological studies (21, 29, 32). It might be worthwhile to validate our results in other countries.

Acknowledgments

References

1. Addiss, D. G., J. P. Davis, B. D. Meade, D. G. Burstyn, M. Meissner, J. A. Zastrow, J. L. Berg, P. Drinka, and R. Philips. 1991. A pertussis outbreak in a Wisconsin nursing home. J. Infect. Dis. 164:704–710.

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

3. Cattaneo, L. A., G. W. Reed, D. H. Haase, M. J. Wills and K. M. Edwards. 1996. The seroepidemiology of *Bordetella pertussis* infections: a study of persons aged 1–65 years. J. Infect. Dis. 173:1256–1259.

4. Cherry, J. D. 1997. The role of *Bordetella pertussis* infections in adults in the epidemiology of pertussis. Dev. Biol. Stand. 89:181–186.

5. Cochran, W. G. 1997. Sampling techniques, 3rd ed. John Wiley & Sons, New York, N.Y.

6. Cromer, B. A., J. Goydos, J. Hackell, J. Mezzatesta, C. Dekker and E. A. Mortimer. 1993. Unrecognized pertussis infection in adolescents. Am. J. Dis. Child. 147:575–577.

7. Deen, J. L., C. A. M. Mink, J. D. Cherry, J. D. Cherry, P. D. Christenson, E. F. Pineda, K. Lewis, D. A. Blumberg and L. A. Ross. 1995. Household contact study of *Bordetella pertussis* infections. Clin. Infect. Dis. 21:1211– 1219.

8. Deville, J. G., J. D. Cherry, P. D. Christenson, E. Pineda, C. T. Leach, T. L. Kuhls and S. Viker. 1995. Frequency of unrecognized *Bordetella pertussis* infections in adults. Clin. Infect. Dis. 21:639–642.

9. Giammanco, A., A. Chiarini , P.A.C. Maple, N. Andrews, R. Pebody, N. Gay, R.M. Ölander, F. Fivet-Groyne, S. Baron, A. Tischer, S. Swidsinski, J. Schellekens and E. Reizenstein. 2003. European sero-epidemiology network: standardisation of the assay results for pertussis. Vaccine 22:112-120.

10. Giammanco, A., S. Taormina, M. Genovese, G. Mangiaracina, G. Giammanco and A. Chiarini. 1997. Serological responses to infection with *B. pertussis*. Dev. Biol. Stand. 89:213–220.

11. Giammanco, A., A. Chiarini, T. Stroffolini, M. Chiaramonte, M. E. Moschen, I. Mura, G. Rigo, S. Taormina, A. Sarzana, G. Mazza and B. Scarpa. 1991. Seroepidemiology of pertussis in Italy. Rev. Infect. Dis. 13:1216–1220.

12. Giuliano, M., P. Mastrantonio, A. Giammanco, A. Piscitelli, S. Salmaso and S. G. F. Wassilak. 1998. Antibody responses and persistence in the two years after immunization with two acellular vaccines and one whole-cell vaccine against pertussis. J. Pediatr. 132:983–988.

13. Granström, G., B. Wretlind and M. Granström. 1991. Diagnostic value of clinical and bacteriological findings in pertussis. J. Infect. 22:17–26.

14. Halperin, S. A., R. Bortolussi and A. J. Wort. 1989. Evaluation of culture, immunofluorescence and serology for diagnosis of pertussis. J. Clin. Microbiol. 27:752–757.

15. Halperin, S. A., R. Bortolussi, A. Kasina and A. J. Wort. 1990. Use of a Chinese hamster ovary cell cytotoxicity assay for the rapid diagnosis of pertussis. J. Clin. Microbiol. 28:32–38.

16. He, Q., J. Mertsola, H. Soini, M. Skurnik, O. Ruuskanen and M. K. Viljanen. 1993. Comparison of polymerase chain reaction with culture and enzyme immunoassay for diagnosis of pertussis. J. Clin. Microbiol. 31:642– 645.

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

18. Jansen, D. L., G. C. Gray, S. D. Putnam, F. Lynn and B. D. Meade. 1997. Evaluation of pertussis in U.S. Marine Corps trainees. Clin. Infect. Dis. 25: 1099–1107.

19. Labadie, J., L. C. Sundermann, H. C. Rümke and the DTP-IPV–Hib Vaccine Study Group. 1996. Multicenter study on the simultaneous administration of DTP-IPV and Hib PRP-T vaccines. Part 1. Immunogenicity. Report 124001003. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

20. Long, S. S., C. J. Welkon and J. L. Clark. 1990. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J. Infect. Dis. 161:480–486.

21. Marchant, C. D., A. M. Loughin, S. M. Lett, C. W. Todd, L. H. Wetterlow, R. Bicchieri, S. Higham, P. Etkind, E. Silva and G. R. Siber. 1994. Pertussis in Massachusetts, 1981–1991: incidence, serologic diagnosis and vaccine effectiveness. J. Infect. Dis. 169:1297–1305.

22. Melker, H. E. de and M. A. E. Conyn-van Spaendonck. 1998. Immunosurveillance and the evaluation of national immunisation programmes: a popula-tion-based approach. Epidemiol. Infect. 121:637–643.

23. Mink, C. M., J. D. Cherry, P. Christenson, K. Lewis, E. Pineda, D. Shlian, J. A. Dawson and D. A. Blumberg. 1992. A search for *Bordetella pertussis* infection in university students. Clin. Infect. Dis. 14:464–471.

24. Müller, F. M., J. E. Hoppe and C. H. Wirsing von König. 1997. Laboratory diagnosis of pertussis: state of the art in 1997. J. Clin. Microbiol. 35:2435– 2443.

25. Nagel, J., S. de Graaf and D. Schijf-Evers. 1985. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LFP antibody levels. Dev. Biol. Stand. 61:325–330.

26. Nelson, J. D. 1978. The changing epidemiology of pertussis in young infants: the role of adults as a reservoir of infection. Am. J. Dis. Child. 132:371–373.

27. Nennig, H. E., H. R. Shinefield, K. M. Edwards, S. B. Black and B. H. Fireman. 1996. Prevalence and incidence of adult pertussis in an urban population. JAMA 275:1672–1674.

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

29. Rosenthal, S., P. Strebel, P. Cassiday, G. Sanden, K. Brusuelas and M. Wharton. 1995. Pertussis infection among adults during the 1993 outbreak in Chicago. J. Infect. Dis. 171:1650–1652.

30. SAS Institute, Inc. 1997. STAT software: changes and enhancement through release 6.12. SAS

Institute, Inc., Cary, N.C.

31. Schmitt-Grohé, S., J. D. Cherry, U. Heininger, M. A. Überall, E. Pineda and K. Stehr. 1995. Pertussis in German adults. Clin. Infect. Dis. 21:860–866.

32. Steketee, R. W., D. G. Burstyn, S. G. F. Wassilak, W. N. Adkins, M. B. Polyak, J. P. Davis and C. R. Manclark. 1988. A comparison of laboratory and clinical methods for diagnosis of pertussis in an outbreak in a facility for the developmentally disabled. J. Infect. Dis. 157:441–449.

33. Storsaeter, J., H. O. Hallander, L. Gustafsson and P. Olin. 1998. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine 16:1907–1916.

34. Tomoda, T., H. Ogura and T. Kurashige. 1991. Immune responses to *Bordetella pertussis* infection and vaccination. J. Infect. Dis. 163:559–563.

35. van der Zee, A., C. Agterberg, M. Peeters, F. Mooi and J. Schellekens. 1996. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples of patients with suspected whooping cough from a highly immunized population. J. Infect. Dis. 174:89–96.

36. Wirsing von König, C. H., H. Rott, H. Bogaerts and H. J. Schmitt. 1998. A serologic study of organisms possibly associated with pertussis-like coughing. Pediatr. Infect. Dis. J. 17:645–649.

Chapter 3

Laboratory-confirmed reinfections with *Bordetella pertussis*

F.G.A. Versteegh [1], J.F.P. Schellekens [2], A.F. Nagelkerke [3], J.J. Roord [3]

1: Department of Paediatrics, Groene Hart Hospital, Gouda, The Netherlands
2: Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment, Bilthoven, The Netherlands
3: Department of Paediatrics, Academic Hospital, Free University, Amsterdam, The Netherlands

Abstract

Susceptibility to infection with *Bordetella pertussis* re-emerges several years after pertussis vaccination. However, the duration of immunity after natural infection with *B. pertussis*, postulated to be lifelong, is not known. In an ongoing study, the longitudinal course of pertussis antibodies in patients who have had laboratory confirmed pertussis is being followed using sera obtained at irregular intervals. In 4 patients a re-infection with *Bordetella pertussis* is described respectively 7 (patient A), 12 (patients B and C) and 3.5 (patient D) y after the first infection. It seems that the longer the interval between the infections the more severe the complaints. *Conclusion:* To the authors' knowledge, these are the first patients in whom symptomatic re-infection with *Bordetella pertussis* has definitely been proven by laboratory confirmation of both episodes. *Bordetella pertussis* infection should be considered in patients with symptoms of typical or atypical whooping cough, irrespective of their vaccination status or previous whooping cough.

Introduction

Bordetella pertussis causes a respiratory infection, which is most severe and potentially fatal in very young children. Most countries have implemented nation-wide vaccination against *B. pertussis*. However, owing to waning of vaccine-induced immunity (1, 2) infections with *B. pertussis* persist in vaccinated populations and these may remain unrecognized because of the atypical or mild nature of symptoms (3–6).

In contrast, it has been commonly accepted that natural infection with *B. pertussis* confers lifelong protection (7, 8). However, this view has been challenged by several reports of occurrences of pertussis in patients who have previously had pertussis (8, 9). In these reports, definite laboratory confirmation that the first episode had indeed been pertussis was lacking. This report describes patients who suffered laboratory-confirmed typical pertussis and subsequently contracted a second episode of coughing which was proven to be caused by *B. pertussis*. They took part in a study of the longitudinal course of pertussis antibodies after natural infection (12).

Case reports

Patient A was born in 1989. For religious reasons her family refused vaccination. She visited our department for the first time in 1991 because of asthma.

In August 1992 she visited the outpatient clinic because of severe paroxysmal coughing. Because of the persisting coughing, pertussis serology was performed and demonstrated a greater than

48-fold rise in IgG antibodies titres to pertussis toxin (table 1) (10). In a later serum also IgA antibodies to *B. pertussis* (11) were detected and IgG antibodies to pertussis toxin had increased further to levels typical of recent infection (12) (table 1). After approximately 10 weeks of severe paroxysmal coughing the patient eventually recovered completely. In serum obtained in 1995, three years after the first pertussis episode, the IgA and IgG antibody levels had decreased to low levels (table 1).

Table 1. Levels of IgA antibody to *B. pertussis* and IgG antibody to pertussis toxin in consecutive sera of patient A; the first symptomatic infection occurred in 1992, the second in 1999.

Date (dd-mm-yy)	Clinical status	IgA (11) (U ml^{-1})	IgG (10, 12) (U ml^{-1})
06-08-92	n.s.	1	1
10-09-92	Paroxysmal cough	2	48
16-11-92	Paroxysmal cough	10	248
30-09-95	n.s.	7	6
31-03-99	Mild coughing	8	260[a]
09-04-99	Mild coughing	14	344
28-04-99	n.s.	20	492
09-09-00	n.s.	7	44

n.s.: no symptoms
[a] Pertussis positive by polymerase chain reaction

In 31 March 1999 she visited the outpatient clinic because of a new episode of coughing, which had started 9 days before. Her pertussis serology showed a high level of IgG antibodies to pertussis toxin suggesting recent infection. Subsequently, PCR (polymerase chain reaction) on material obtained from a nasopharyngeal swab demonstrated the presence of *B. pertussis* (13). She fully recovered in less than 3 weeks. The follow-up of pertussis serology showed that IgA and IgG antibody levels reached a peak at 5 weeks after onset of symptoms and that 9 and 16 months later these levels had considerably decreased again.

Patients B and C are female twins born in 1984. They were vaccinated against pertussis in the first year of life. From the age of one year they came under pediatric attention because of allergic rhinitis and severe bronchial asthma. In September 1989 they visited the outpatient clinic because of a change in cough pattern which reminded their grandmother of whooping cough. Two to three weeks before the visit they suffered for one or two days from severe coughing and high fever. In patient B this was accompanied by vomiting, in patient C by nasal discharge and diarrhea. In the weeks after they had cough paroxysms, especially during the night, often followed by vomiting and an inspiratory "whoop" at the end of a paroxysm. Paired serum analysis showed a significant rise of IgA and IgG titers against *B. pertussis* in patient B (table 2). In patient C titres did not rise significantly but they met the criteria for the diagnosis of *B. pertussis* infection with one point serology.

Table 2. Levels of IgA antibody to *B. pertussis* and IgG antibody to pertussis toxin in consecutive sera of patient B and C; the first symptomatic infection occurred in 1989, the second in 2001.

Date (dd-mm-yy)	Clinical status	Patient B		Patient C	
		IgA (11) (U ml $^{-1}$)	IgG (10, 12) (U ml $^{-1}$)	IgA (11) (U ml $^{-1}$)	IgG (10, 12) (U ml $^{-1}$)
20-09-89	Paroxysmal cough	1	1	1	72
10-10-89	Paroxysmal cough	8	88	6	144
27-10-89	Paroxysmal cough			9	112
11-06-01	Paroxysmal cough	68	368		
01-07-01	Paroxysmal cough			38	168

The symptoms gradually faded during the next 4 months. In patient C a third titre was determined two weeks after the second, which showed a further increase in IgA and a decrease in IgG titre. Between 1989 and 2001 no blood samples came available for this study. In June 2001 both girls again visited the out patient clinic because of a cough, which lasted for four weeks and reminded their father of their first whooping cough episode. Patient B had experienced several nightly attacks with a whooping type of cough resulting in shortness of breath, which gave her the feeling of near suffocation. After several nights she learned to cope better with the attacks, which she thought to be caused by hyperventilation. In patient C the course was milder (cough attacks followed by a whooping inspiration). One point serology against *B. pertussis* was positive in both girls. Symptoms disappeared spontaneously in about two months.

Table 3. Levels of IgA antibody to *B. pertussis* and IgG antibody to pertussis toxin in consecutive sera of patient D; the first symptomatic infection occurred in 1998, the second in 2001. In February 2001 she suffered from Influenza A.

Date (dd-mm-yy)	Clinical status	IgA (11) (U ml $^{-1}$)	IgG (10, 12) (U ml $^{-1}$)
13-05-98	Nocturnal coughing and common cold	11	228
26-05-98	Nocturnal coughing	39	500
02-09-99	n.s.	25	71
01-02-01	Mild coughing	11	10
16-02-01	Mild coughing	14	10
07-11-01	Coughing now and then	27	492

n.s.: no symptoms

Patient D was born in 1994 and was regularly vaccinated. She was referred to the outpatient clinic because of persisting cough with common cold and tightness of the chest in May 1998. She was already treated for 2 weeks with clarithromycin and anti-asthmatic drugs without success. Serum analysis showed a significant rise of IgA and IgG titers against *B. pertussis* (table 3). Continuation of therapy finally resulted in complete recovery within another month. She was then followed for her asthma. In February2001 she suffered again from coughing during an Influenza A (H1N1) infection. Titers against *B. pertussis* were low. She recovered within 2

weeks. In November 2001 she came for a routine visit. Her brother was diagnosed to have a *B. pertussis* infection in August 2001. She only suffered from a little coughing now and then, not worth mentioning, starting early September. Serology was again positive for *B. pertussis*.

Discussion

Little is known about the persistence of immunity to *B. pertussis* after natural infection. The high levels of IgG antibodies to pertussis toxin (IgG-PT) that develop after natural infection decline to low levels within a few years (12), as typified in patients A and D. However, although high levels of IgG-PT are associated with protection (14), no evidence has been available that links low levels of these antibodies with susceptibility. In patients A and D, 4y after the first episode, the IgG-PT had declined to low levels (Tables 1 and 3) but at the time of the second infection (7 and 3.5 y after the first infection) partial immunity was still present, as suggested by the short duration, mild nature of symptoms and the rapidity of the humoral immunoresponse compared with the first episode. Whether or not the relatively short interval in both patients between first and second symptomatic infection episodes is exceptional cannot be ascertained, since no other data on proven re-infection could be found.

Others have concluded that natural infection does not provide lifelong protection against whooping cough as there are cases of re-infection based on clinical history but without laboratory confirmation (8, 9). Theoretically, other infectious agents could have been the cause for the recurrent pertussis syndrome (15). In most cases of possible re-infection the recalled pertussis syndrome had occurred at least 20 y earlier (9).

To the authors' knowledge, these cases are the first in which both the first and the second infection episodes were clinically documented and confirmed with laboratory tests. The fact that the patients participated in a long-term follow-up study of antibody levels after infection with *B. pertussis* (12) probably played an important role in detecting the second infection. It is very doubtful whether the second episode in patients A and D would have come to medical attention or would have been diagnosed as pertussis outside the confines of the study.

The expected persistence of a reservoir of *B. pertussis* in the population underscores the need for the development of alternative strategies specifically to protect unvaccinated neonates against potentially fatal pertussis. For example, booster vaccinations repeated throughout life may be considered (16).

The immediate implication of this observation is that patients who present with symptoms compatible with typical or atypical pertussis should be considered to have pertussis, not only if they have been previously vaccinated but also if they have had whooping cough before.

References

1. Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study. Br Med J 1988; 296: 612–4

2. Black S. Epidemiology of pertussis. Pediatr Infect Dis 1997; 16: S85–9

3. Melker HE de, Conyn-van Spaendonck MAE, Rümke HC, Wijngaarden JK van, Mooi FR, Schellekens JFP. Pertussis in the Netherlands: an outbreak despite high levels of immunization with whole-cell vaccine. Emerging Infect Dis 1997; 3: 175–8

4. Deeks S, De Serres G, Boulanne N, Duval B, Rochette L, Déry P, et al. Failure of physicians to consider the diagnosis of pertussis in children. Clin Infect Dis 1999; 28: 840–6

5. Long SS, Lischner HL, Deforest A, Clark JL. Serologic evidence of subclinical pertussis in immunized children. Pediatr Infect Dis J 1990; 9: 700–5

6. Strebel P, Nordin J, Edwards K, Hunt J, Besser J, Burns S, et al. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995–1996. J Infect Dis 2001; 183: 1353–9

7. Bass JW. Erythromycin for treatment and prevention of pertussis. Pediatr Infect Dis 1986; 5: 154–7

8. Laing JS, Hay M. Whooping cough: its prevalence and mortality in Aberdeen. Public Health 1902; 14: 584–98

9. Wirsing von König CH, Postels-Multani S, Bock HL, Schmitt HJ. Pertussis in adults: frequency of transmission after household exposure. Lancet 1995; 346: 1326–9

10. Nagel J, Graaf S de, Schijf-Evers EJ. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG antiLPF antibody levels. Dev Biol Standard 1985; 61: 325–30

11. Nagel J, Graaf S de, Schijf-Evers EJ. Serum IgA antibody to *Bordetella pertussis* as an indicator of infection. J Med Microbiol 1983; 16: 417–26

12. Melker HE de, Versteegh FGA, Conyn-van Spaendonck MAE, Elvers LH, Berbers GAM, Zee A van der, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. J Clin Microbiol 2000; 38: 800–6

13. Zee A van der, Agterberg C, Peeters M, Mooi F, Schellekens J. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis 1996; 174: 89–96

14. Long SS, Welkon CJ, Clark JL. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis 1990; 161: 480–6

15. Wirsing von König CH, Rott H, Bogaerts H, Schmitt HJ. A serological study of organisms possibly associated with pertussis-like coughing. Pediatr Infect Dis J 1998; 17: 645–9

16. Hethcote HW. Simulations of pertussis epidemiology in the United States: effects of adult booster vaccinations. Math Biosci 1999; 158: 47–73

Chapter 4

Kinetics of the IgG antibody response to pertussis toxin after infection with *Bordetella pertussis*

P.F.M. Teunis [1], O.G. van der Heijden [2], H.E. de Melker [2],
J.F.P. Schellekens [3], F.G.A. Versteegh [4], M.E.E. Kretzschmar [2]

1: Computerization and Methodological Consultancy Unit, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

2: Centre for Infectious Diseases Epidemiology, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

3: Laboratory of Infectious Diseases Screening, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

4: Groene Hart Ziekenhuis, Department of Pediatrics, Gouda, The Netherlands

Summary

We aimed to provide a quantitative description of decay in pertussis antibody levels to aid in finding a serological estimate of the incidence of pertussis. The serum IgG response against pertussis toxin was studied in a group of clinically diagnosed patients. Individual records consisted of repeated serum IgG measurements at irregular intervals for up to 10 years post diagnosis. These data were analysed with a nonlinear regression model taking into account censoring at upper and lower threshold levels, measurement errors and individual variation in the shape and magnitude of the immune response. There was considerable variation between individual responses, both in strength (amplitude) and duration (shape). The inverse model relating IgG levels to time from infection (diagnosis) can be applied to cross-sectional IgG data to generate distributions of times from infection, which may be used to calculate infection rates and their variation, in populations sampled for cross-sectional IgG data.

Introduction

Pertussis (whooping cough) is an infection of the respiratory tract caused by the highly contagious bacterium *Bordetella* (*B.*) *pertussis*. Despite high vaccination coverages, *B. pertussis* is still circulating in the Dutch population. Notification data show that most symptomatic cases occur among vaccinated children aged 4–9 years [1]. However, symptoms are most severe among infants who are too young to be vaccinated. In order to protect this group at risk it is important to determine which people are most likely to transmit infection to newborns. We aimed to identify those age groups in the population in which most of the circulation of *B. pertussis* takes place. The age profile of notified cases may not reflect the age distribution of infection with *B. pertussis* because the case/infection ratio is probably higher in younger age groups [2]. It was necessary to determine the infection rates in each age category, rather than notification rates.

In response to an infection, IgG titres typically show a rapid increase, followed by a steady, slow decline over a long period (several years) [1, 3–9]. Also IgG-PT induced after three or four doses of acellular or whole cell pertussis vaccine in the first year of life declines rapidly, mostly within one year, to low levels [10, 11]. Therefore, it is likely that in individuals in whom the last vaccination with pertussis vaccine has been administered more than one year ago, the finding of moderate or high levels of IgG-PT indicates infection with *B. pertussis*. Because the magnitude of the IgG-PT level is inversely related to time elapsed since infection, this suggests that in a patient with a given response, time from infection can be estimated from IgG levels. If this approach to estimating times from infection were feasible, cross-sectional studies of IgG-PT-levels could be used to estimate the incidence of infection independent of case

notification. This requires quantitative characterization of the IgG-PT-response to pertussis inclusive of its variation among individual patients.

We used data from an ongoing long-term follow-up study in which blood samples were taken at irregular intervals from patients with clinically and serologically confirmed pertussis. At present, this study comprises 85 patients with follow-up times ranging from 6 months to 11 years. Numbers of samples were unequally distributed among patients. In an earlier report on the first 57 patients in this longitudinal study it was shown that despite large variation in responses the general pattern appeared to be a rapid increase in antibody levels followed by a slow decrease.

We tested several mathematical functions for their ability to fit these observed changes in IgG-PT levels with time. Here we present results of this analysis taking into account censoring at upper and lower threshold levels, measurement errors, as well as individual variation in the shape and magnitude of the IgG-PT immune response.

The selected model describes a functional relationship between time since last infection and level of antibody titre. The model was applied to a cross-sectional population based study of IgG-pertussis antibody titres, permitting estimation of a distribution of infection dates for individuals in this population. Such model-based age-specific distributions of times since infection assist in identifying those age groups in which circulation of *B. pertussis* is most prevalent.

Methods

Data used

During the period 1989–2000, a collection of follow-up serum samples was obtained from 85 patients clinically diagnosed with pertussis (paroxysmal cough lasting more than 2 weeks) in whom the clinical diagnosis had been confirmed by the finding of an IgG-PT level of 75 U/ml in the first or the second serum obtained in the symptomatic stage. The specificity of IgG-PT of 75 U/ml as an indicator of recent infection with *B. pertussis* has been estimated to be >97.5 % while the sensitivity was around 80 % [1]. For participation in this study a minimum follow-up period of 3 months was required.

In one of the participating patients a second symptomatic infection with *B. pertussis* occurred 7 years after the first, confirmed by positive pertussis PCR and a strong rise of IgG-PT. For analysis in this study we considered this record to be two patients: the first one connected to the first episode of pertussis and its follow-up until the last sampling before the second episode and one connected to the second episode and the follow-up thereafter. Thus, in 85 patients, 86 episodes of pertussis and the course of IgG-PT thereafter were analysed. Recently, an additional three patients appeared to be re-infected [12]. These were not included in the present analysis.

The follow-up period ranged from 6 months to 11 years and the number of serial sera per patient ranged from 2 to 11. The age distribution of the patients is given in Figure 1. Most were children between 0.5 and 17 years of age (69/86). Eleven infants were less than 6 months old at the time they contracted pertussis. Also included were six adults with ages ranging from 30 to 41 years. Vaccination status as reported by the physician at the time of onset of pertussis was in 1 of 4 categories: negative, incomplete (1 or 2 vaccinations), complete (3 or 4 vaccinations) or unknown. Four infants whose vaccination had not been completed at the time of pertussis were vaccinated shortly thereafter. We therefore decided to use five categories of patients: infants (0–0.5 years) vaccinated after infection (4); infants not vaccinated after infection (7); vaccinated juveniles (0.5–20 years) (62); unvaccinated juveniles (7) and adults (6). These subgroups were analysed separately.
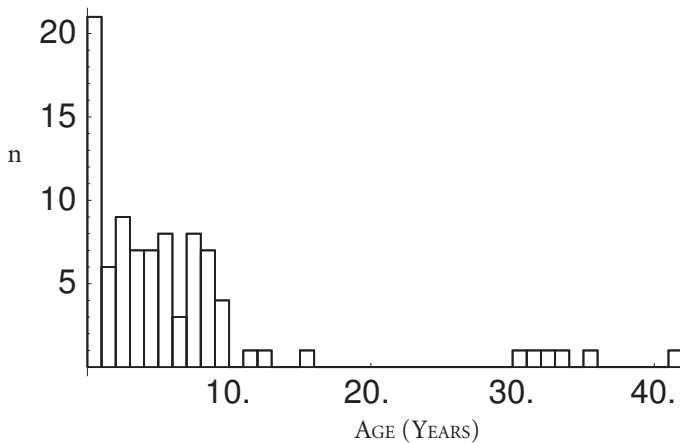
Fig. 1. Age distribution of patients.

Only changes in the highly specific anti-pertussis IgG-titres were analysed. Titres were determined by enzyme-linked immunosorbent assay (ELISA) [11, 13] expressed in arbitrary units (' Dutch units per ml '). In Figure 2 all measurements from all included patients are shown. Reproducibility of measurements was checked according to Standard Operating Procedure (SOP: coefficient of variation in log-transformed readings from three control sera (low, medium and high titre) less than 20 %). For quantitative analysis, the scatter in these measurements therefore was considerable.

Below 5 U/ml the ELISA test is considered insensitive, therefore 5 U/ml was interpreted as 5 U/ml or less. Serial dilutions were used to an upper limit corresponding to a concentration of 500 U/ml: a value of 500 U/ml was therefore interpreted as 500 U/ml or higher.
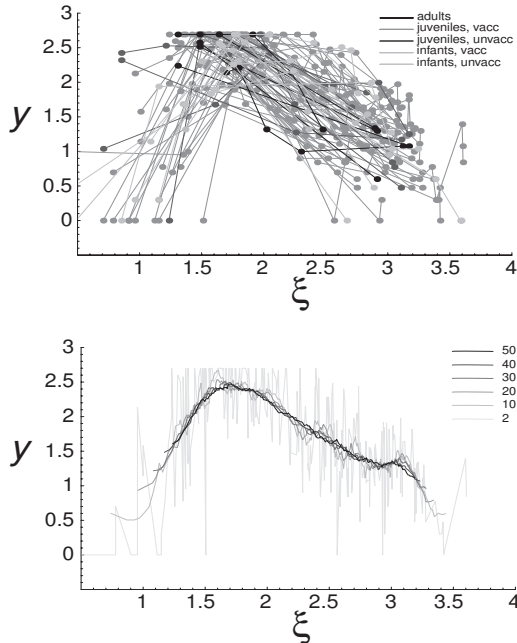
Fig. 2. Data set of log-transformed IgG-values against log time (in days) from all patients diagnosed at time 0 with symptomatic infection by *Bordetella pertussis*. Individual responses are connected, and log-transformed responses of vaccinated juvenile patients against log-time, after application of a smoothing kernel (moving average, bandwidth as in inset), showing linear increase and decrease.

Each patient record started with the time the first symptoms occurred. Strictly speaking, we did not estimate times from infection but times from first symptoms (or even first diagnosis). Since incubation periods may show variation among individual patients, individual responses could have been slightly offset relative to each other.

Response model

During the decreasing phase, the change in IgG titre with time appeared to be less steep than an exponential decay function. In a log–log graph, an exponential relation is a convex function with increasing negative slope with log-time. Our data did not seem to indicate such behaviour. Instead, in a log–log graph, decay appeared to be more or less linear (Fig. 2). This called for a power function as a model, which on a log–log scale would be a straight line with arbitrary slope. However, observations were taken at random points in time, usually starting somewhere during the rising phase of the response. Fitting a straight line to the log– log transformed data, as reported by de Melker [14], only accounted for the decaying phase of the response and required omission of these initial observations. Since there was no clearly defined criterion by which observations would be excluded, we chose to use a response model

that included the initial rising phase.

Let $g(t;\boldsymbol{\theta})$ denote the IgG-response on a linear scale and $f(\xi;\boldsymbol{\theta})$ its $\log_{10}$ as a function of log time $\xi = \log_{10}(t)$ with parameter vector $\boldsymbol{\theta}$

$$f(\xi;\boldsymbol{\theta}) = d + c\left[\xi + b\left(1 - \sqrt{1 + \frac{\xi^2}{a}}\right)\right] \dots\dots\dots\dots\dots\dots\dots(1)$$

is a skewed hyperbola with linear asymptotes with arbitrary slopes for $\xi \to -\infty$ and $\xi \to \infty$, respectively and parameter vector $\boldsymbol{\theta} = (a,b,c,d)$

For $\xi \to -\infty$ and $\xi \to \infty$ this function approaches the asymptotes

$$\lim_{\xi \to \mp\infty} d + bc + c\xi\left(1 \pm \frac{b}{\sqrt{a}}\right) - f(\xi;\boldsymbol{\theta}) = 0 \dots\dots\dots\dots\dots\dots (2)$$

The parameter d can be interpreted as the amplitude of the response and the parameter a mainly determines the long-term decrease of the response.

Figure 3 illustrates the shape of this function, in a non-transformed (lin–lin) graph to show the very slow decline with time.

Model-fitting procedure

The response model [equation (1)] can be used to calculate expected values of the logarithm of the IgG titre.
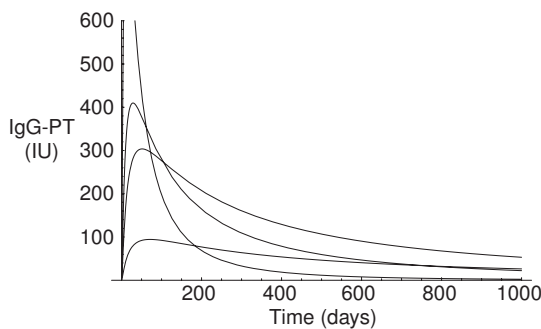

Fig. 3. Representative individual response curves generated by the model in equation (1), fitted to data from vaccinated juvenile patients. These curves illustrate characteristics of the responses: rapidly rising titres, followed by very slow decrease, and variation in both levels and steepness of the curves.

Assuming normally distributed measurement errors, with standard deviation σ, the log-likelihood function can be written as

$$L_1(\boldsymbol{\theta}, \sigma) = \sum_i \log(\varphi[X_i; f(\Xi_i; \boldsymbol{\theta}), \sigma]) + \sum_j \log(\Phi[Y_j; f(\Xi_j; \boldsymbol{\theta}), \sigma]) +$$

$$\sum_k \log(1 - \Phi[Z_k; f(\Xi_k; \boldsymbol{\theta}), \sigma]) \qquad \qquad \text{......(3)}$$

with $\varphi[X_i; f(\Xi_i; \boldsymbol{\theta}), \sigma]$ a normal probability density function (expected value $f(\Xi_i; \boldsymbol{\theta})$, standard deviation σ) for the contribution of a measured log-titre X_i at log-time Ξ_i. For a log-titre Y_j equal to, or below the lower measurement threshold, the contribution is given by the cumulative normal distribution function $\Phi[Y_j; f(\Xi_j; \boldsymbol{\theta}), \sigma]$. For a measurement Z_k equal to or above the upper threshold, the appropriate contribution is $1 - \Phi[Z_k; f(\Xi_k; \boldsymbol{\theta}), \sigma]$. In our case the lower threshold in the ELISA test was 5 U/ml and the upper threshold was 500 U/ml. Parameter values of the response model (*a, b, c, d*) and the error of the detection method would be determined using this likelihood function.

Initially, our response model was fitted to the pooled data neglecting individual variation. In this 1-level model measurement error was the only possible source of uncertainty. In order to assess variation in responses among individual subjects we would have fitted our model to the measurements of each individual response, thereby generating sets of parameters $(\boldsymbol{\theta}_i, \sigma_i)$ for every individual *i* in the population. Since individual responses often consisted of only a few measurements, we decided to allow only one or two parameters to vary among individual subjects. Leaving aside the measurement error parameter σ, fitting a model equation with four parameters to a population of *n* subjects thus generates *n+3* parameter values (or *2n+2*, where two parameters vary among individual responses), when 3 (2) parameters are equal for all subjects and *n (2n)* estimates for the (third and) fourth parameter to describe variation among subjects.

In our response model in equation (1), the parameters *d* describing the vertical offset on a log-scale (or the amplitude of the response) and *a* describing the shape of the response could be employed to model variation among individuals; the parameters *b* and *c* were shared by the whole population. The parameter σ (the measurement error) was assumed to be independent of the individual whose serum was being analysed.

Therefore, our final model included individual variation in both *a* and *d*, leaving us with a set $\{(a_1, \cdots, a_n), b, c, (d_1, \cdots, d_n), s\}$ to be determined by optimization of the likelihood function

$$L_2[(a_1, \cdots, a_n), b, c, (d_1, \cdots, d_n), \sigma] = \sum_{i=1}^n L_1(a_i, b, c, d_i, \sigma) \ldots \ldots \ldots \ldots (4)$$

In order to avoid cumbersome likelihood optimization procedures this two-level model was analysed by a Markov chain Monte Carlo (MCMC) method, using the Metropolis–Hastings algorithm [15]. Parameters were log-transformed, initial values were set at the values found for the one-level model (individual values for $\log(a_i)$ and $\log(d_i)$ all equal) and prior distributions for the log-transformed parameters were uniform probability distributions with an interval of (-3, +3) log-units about the initial value (wider intervals were checked and did not produce different results). For each iteration in the Markov chain the likelihood value was tabulated. The model was then allowed to run for about 10 000 iterations and a trend test was used to confirm stationarity of the series of likelihood values (equal means in successive bisections of the series). The iteration with the highest likelihood value was then chosen from this set of likelihood values

$$L_2\left[\left(\hat{a}_1,\cdots,\hat{a}_n\right),\hat{b},\hat{c},\left(\hat{d}_1,\cdots,\hat{d}_n\right),\hat{\sigma}\right]=\max_{j\in 1,\cdots,m} L_2\left[\left(a_{1,j},\cdots,a_{n,j}\right),b_j,c_j,\left(d_{1,j},\cdots,d_{n,j}\right),\sigma\right] \cdots\cdots\cdots(5)$$

This was taken as an approximation of the true maximum likelihood parameter set. When the resulting response functions were plotted against separate individual observed responses, this seemed to be a reasonable assumption. The likelihood values also appeared to fall well below the values found for the one-level model and those of alternative two-level models with either *a* or *d* varying among individuals. Compared to a 'likelihood supremum' obtained by using the observed tires instead of the model function (using the measurement error from the fitted model) a significant improvement in goodness of fit was found (likelihood ratio tested against χ^2 deviate). This can be explained by the apparent lack of fit early in the responses (Fig. 5a). It should be noted that when individual responses were fitted, each response curve was based mostly on only a few (sometimes as few as three) observations. Therefore, the χ^2 approximation of the deviance function should be treated with caution.

For a sequence of log-times 5, 50 and 95% percentiles were determined for these maximum likelihood fits. The median curve was used to describe the time–titre relation, while the 5 and 95% percentiles indicated the magnitude of the variation among members of this population.

In addition to the maximum likelihood estimates, the Monte Carlo method provided information on parameter uncertainty, as the Markov chains for all parameters could be regarded as a sample from their joint posterior distribution.

Patients were categorized according to age (adults older than 20 years, infants younger than 6 months and juveniles older than 6 months and younger than 20 years) and vaccination status (unvaccinated and vaccinated). To test whether the two categories could be merged, given a certain regression model, a likelihood ratio test was used as follows: first, calculate maximum likelihood values for each separate data category (say L_a and L_b). Then, pool the data and maximize the likelihood for the merged data (yielding a log-likelihood L_{ab}).

The difference $-2(L_{ab} - L_a - L_b)$ would now be tested against a χ^2 variate with degrees of freedom equal to the number of parameters of the regression model [16]. Various combinations of categories were tested using this method.

Results

Table 1 summarizes the results of application of the two-level model to the various patient categories (age, vaccination status). In Table 2 results of the likelihood ratio test for merging various combinations of categories are given. None of the response categories could be merged; differences were significant for all the tested combinations. Given the applied model, the IgG-PT response to infection with *B. pertussis* was seen to change with age and with vaccination status.

Table 1. *Maximum log-likelihoods for the two-level model applied to separate patient categories*

	No. patients	One-level		Two-level	
		No. par*	$-2 \log(L)$	No. par	$-2 \log(L)$
Juv, vacc	62	5	536·4	127	434·0
Juv, unvacc	7	5	48·9	17	33·6
Adults	6	5	25·0	15	6·6
Infants, vacc	4	5	10·5	11	0·5
Infants, unvacc	7	5	44·5	17	4·6

* No. par = number of parameters.

Table 2. *Maximum log-likelihoods for joint categories, and differences $\Delta[-2 \log(L)]$ with separately fitted model. The last column shows the significance of the difference (likelihood ratio test, as explained in the text, model fitting procedure) at the 0·95 level. Therefore, responses from all categories must be considered different, given the proposed model*

	(log)likelihood		(log)likelihood ratio		
	D.F.	$-2 \log(L)$	ΔD.F.	$\Delta[-2 \log(L)]$	Significance
Juv, vacc + unvacc	141	486·0	3	18·4	+
Juv, vacc + unvacc, adults	153	524·0	6	49·7	+ +
Infants, vacc + unvacc	25	17·8	3	12·7	+
All	175	588·2	12	113·1	+ +

Figure 5 shows two-level model fits to individual responses, illustrating large variations in shape and amplitude of responses in individual patients.

Although statistically significant, the differences due to vaccination status within the two youngest age categories, infants and juveniles, were not large (Figs 5 a, b and 5 c, d, respectively). A small group of adult patients (Fig. 5e) appeared to have a response that differed from the

younger patients. The most prominent difference was the lack of early measurements: these adult patients apparently visited their physician later than the younger patients. This may have been associated with milder symptoms in adults (for instance causing a parent to infect a child who then developed severe enough symptoms for the infection to be detected in both the parent and the child). The decay of the response in these adults did not differ greatly from that of the vaccinated juveniles.
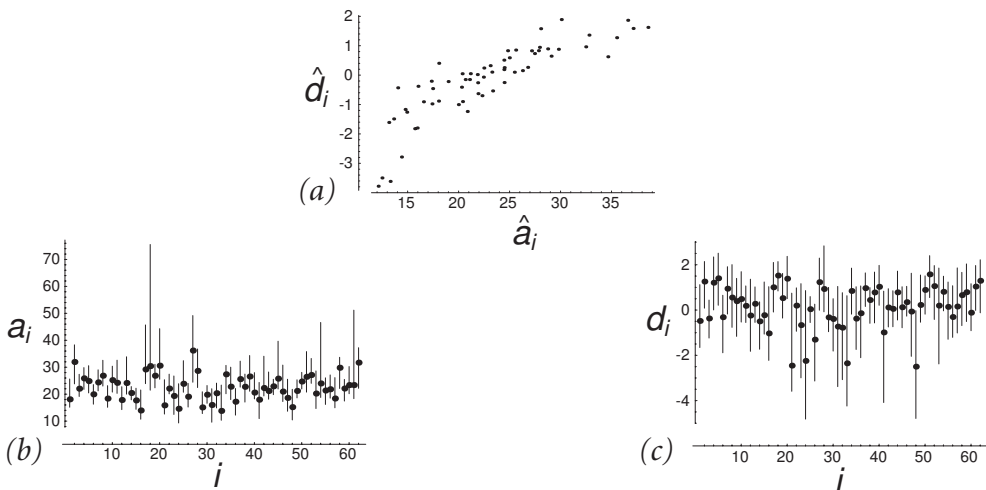

Fig. 4. (*a*) Maximum likelihood values of individual shape parameters ($\hat{a}_i$) and amplitude parameters ($\hat{d}_i$) for the two-level model fitted to vaccinated juvenile patients. (*b*) Variation in shape (parameter a_i) among patients (rank number *i*, random order) expressed by plotting MCMC-based median values and 0·05 and 0·95 quantiles. (*c*) Variation in amplitude (parameter d_i) among patients expressed by plotting MCMC-based median values and 0·05 and 0·95 quantiles.

Table 3 and Figure 4 show estimated parameter values for the largest category, vaccinated juvenile patients (comprising 62 patients, 72% of all patients). Maximum likelihood parameter values (with median; 5%, 95% percentiles) are shown in Table 3. For parameters a_i (variation in shape of the response) and d_i (variation in amplitude of the response), fitted parameter values for all patients in this category are shown in Figure 4. The uncertainty in these parameters is illustrated in Figure 4 b, c, which show median values with (MCMC-based) 95 % intervals.

Measurement error
In the one-level model, all variation is interpreted as measurement error. Therefore, the value of the parameter σ is larger than in the two-level model, where part of the variation is attributed to heterogeneity in patient responses. Despite the considerable individual variation, the estimated measurement error is very large. In the vaccinated juvenile patients, the logarithm of this error factor decreased from 0.62 in the one-level model to 0.46 in the two-level model. This would mean that a 95 % confidence interval for the IgG-levels would extend over

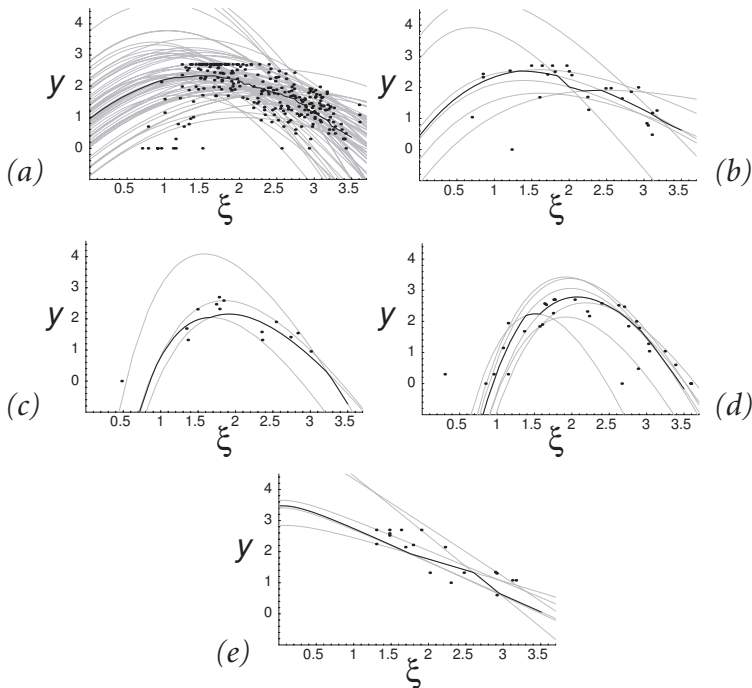approximately a factor five up or down, for the two-level model.

Fig. 5. (*a*) Log–log graph of individual responses of the two-level model, fitted to data from vaccinated juvenile patients. Also shown data and (heavy line) median response. (*b*) Log–log graph of individual responses of the two-level model, fitted to data from unvaccinated juvenile patients. Also shown (*c*) infants, vaccinated after infection, (*d*) infants, not vaccinated after infection, and (*e*) adults.

Table 3. *Parameter estimates for the one-level (1-l) and the two-level (2-l) model, applied to responses from patients in all categories*

	$\hat{a}$		$\hat{b}$		$\hat{c}$		$\hat{d}$		$\hat{\sigma}$	
	1-l	2-l	1-l	2-l	1-l	2-l	1-l	2-l	1-l	2-l
Juv, vacc	22·8	Fig. 4	14·0	15·7	2·68	1·90	1·00	Fig. 4	0·65	0·56
Juv, unvacc	2·15	—	2·00	2·66	6·85	3·09	2·86	—	0·61	0·46
Adults	0·12	—	1·87	4·23	0·25	0·11	−2·81	—	0·45	0·32
Infants, vacc	1·51	—	1·46	1·58	22·3	19·5	13·5	—	0·34	0·22
Infants, unvacc	2·59	—	2·08	2·29	17·2	18·9	11·4	—	0·54	0·31

Time since infection as a function of titre

In order to describe the time elapsed since infection (numbers of days since the appearance of symptoms) as a function of titre, we determined the inverse function of (1).

Given the short duration of the rising part of the response, we considered only the decreasing part of the response. Each response curve had a maximum titre, dependent on the parameter values for an individual patient. Any titres higher than this maximum therefore had no corresponding

time since infection. On a log scale ($y = \log_{10}(\text{IgG titre})$), the inverse relation was

$$h(y;a,b,c,d) = \frac{b\sqrt{a}\sqrt{ac^2 + (d-y)(2bc+d-y)} - a(bc+d-y)}{c(b^2-a)}$$

with $y < d + bc - c\sqrt{b^2 - a}$

This inverse function described (log-)time since last infection as a function of (log-)titre. Figure 6 shows that this inverse function was also subject to considerable individual variation. Given a certain measured IgG-level we now estimated the (variation in) time elapsed since infection.

When doing this, we took into account the fact that not every individual response reached the same maximum IgG titre. Therefore, with IgG titres rising above a certain level, the inverse function did not exist for an increasing number of patients. This is shown in Figure 7a, where the fraction of the individual responses (of vaccinated juvenile patients) that reached a given log-titre *Y*, is shown as a function of *Y*.

Figure 7b shows histograms of the times from infection for a range of IgG-titres, constructed from the inverse responses in Figure 6. Returned estimates were based on decreasing numbers of responses: only those that reached the level of interest were used. Some technical details are given in the Appendix.
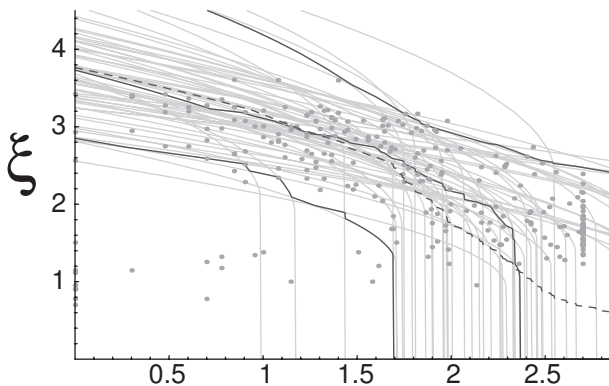
Fig. 6. Expected log-time since infection (ξ) as a function of anti-pertussis log-IgG titre (*y*). Inverse function of the two-level regression model, using maximum likelihood values for all parameters. Also shown (heavy lines) median and 95 % range, and (hatched) arithmetic mean response. Each light grey curve represents an individual response, with its own maximum level. Titres higher than this individual maximum cannot be reached and the inverse response jumps to zero.
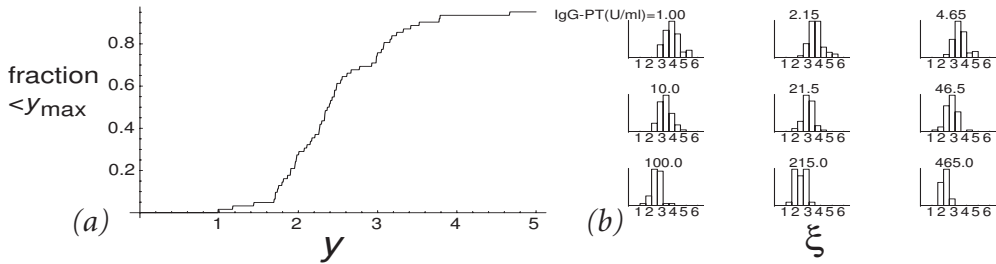
Fig. 7. (*a*) Fraction of the patients (vaccinated juveniles) who failed to reach a level *y* (log-titre). Even at low titres a considerable proportion of the patients do not reach that level and cannot contribute to the estimated time since infection. (*b*) Variation in log-time since infection (ξ) for various levels of anti-pertussis IgG titre.

Discussion

The serum IgG-response in patients diagnosed with pertussis can be described by a simple parametric model but variation among subjects is a prominent phenomenon that must be included in the analysis. IgG titres rise steeply after infection to reach a maximum within a few weeks and then decline slowly over several years. Although this is probably true in any infected subject in a population, incidental measurement of a single IgG titre gives little information about the time since infection. In particular, maximum IgG titres appear to show considerable heterogeneity; a modestly enhanced IgG titre of 20 IU/ml may correspond to very recent infection, or may equally correspond to infection several months earlier, depending on the particular response of the patient. Current practice of characterizing population immune responses with their geometric mean titres neglects the importance of heterogeneity [8, 9, 17]. If the group of patients in the study used here is representative of the general population, at least with respect to their IgG response to pertussis toxin, our results may be employed to estimate times from infection from cross-sectional samples of IgG-PT titres. Any given (measured) IgG-PT titre may thus be assumed to correspond with a time from infection (first diagnosis). Our results also indicate the existence of considerable variation among individual responses. With increasing IgG-PT levels, there also seems to be an increasing fraction of subjects who do not reach that level at any time during their post-infection response. High IgG-PT levels therefore indicate recent infection but only in a small part of the population.

Data set
To apply the results of this analysis to the estimation of incidences of pertussis in the general population, the observed responses should be representative of the responses that would be found in a random sample of this general population. The age distribution in our test population differed substantially from that of the population at large, with juveniles aged less than 20 years strongly overrepresented. Older subjects were hardly present. The heterogeneity

among all patient categories in this study complicates the use of these results for back-calculation of infection from a cross-sectional sample of a completely different population. Estimation of times from infection only involved the decaying phase of the response, where differences may have been least marked. This would easily be tested in a practical application, which we intend to report shortly.

Newborns may be important for illness burden and they should possibly be treated separately when back-calculating times from infection. Unfortunately, we had responses from only a few of these patients but since infection cannot have occurred before birth, time from infection was limited in these infants.

Recorded responses started at the first date of symptoms, as reported by the patients (or their parents). In very young children this may have been earlier than in older patients because of the severity of symptoms. On the other hand, in newborns symptoms may be non-specific, making diagnosis diffcult or delaying diagnosis. An alterative model employing a variable shift in onset (at the expense of yet another parameter per patient) only produced minor offsets indicating that the data offered little support for such differences. Nevertheless, the difference in response shapes between infants and adults (Fig. 5c–e) is striking and seems to support an effect of late onset of symptoms.

The patients included in this study all presented symptoms of respiratory illness. Asymptomatic or mild infection is probably more frequent in adults and could be associated with a different IgG response: a smaller amplitude, or different time course. No information was available to test this hypothesis. Most of the juvenile patients also suffered from chronic respiratory symptoms (asthma); it remains unclear whether this condition increases the susceptibility to infection or could lead to a different immune response to pertussis. Are younger subjects more susceptible to infection, or is there a higher probability that infection is symptomatic [18, 19]? Recently, an animal model with infection and clinical symptoms similar to those in humans has been described [20]. It is conceivable that such a model could be utilized to study details of the differences in susceptibility between adults and infants.

Regression procedure

Heterogeneity in immune responses has been studied for various pathogens. In order to assess immunity, Gay used mixture models to describe the variation in age stratified IgG levels against parvovirus B19 [21]. Hierarchical Bayesian models have been used to describe the decline in immunity and its variation in hepatitis B [15] and *Haemophilus influenzae* [22, 23]. These models assumed linear decline of IgG titres (on a log scale). The immune response against hepatitis A vaccine has been studied by van Herck and colleagues [17, 24]. They studied the decline in geometric mean antibody titre of their subjects with time but also reported on individual responses, presenting evidence of considerable variation among subjects [17].

Since our data included not only the decline but in many cases also the rising slope of the immune response, we needed a model that accommodated both parts of the response, necessarily a non-linear model. Our data also contained strong evidence of censoring; in accounting for this, the fitted responses were steeper than they would have been without correction for censoring.

Part of the variation in the measurements appeared to be explained by individual variation, in amplitude and/or in shape (descending slope) of the response, rather than by some measurement error. In addition to this, other parameters, like incubation period, could also have contributed to the heterogeneity of the responses. We investigated this by incorporating individual variation in shift along the time axis (not shown here). However, this did not result in significant improvement in goodness of fit (judged by log-likelihood); estimated delay times were also very small and appeared to vary little among individual patients.

Biological interpretation of the hyperbola model

The extremely slow decline in IgG antibody titres with time following infection, probably associated with long-term protection, precludes use of a first order model of antibody decay. Such a model would lead to a convex curve, with an increasing downward slope on a log time–log titre scale. For our data, such a slope is much too steep, with poor fit to the measured responses. This may not be always a problem. Tiru et al. [25] reported successful use of a simple exponential model to describe the immune response to diphtheria toxin. The model we used had asymptotically linear decline on a log–log scale at long times from infection.

Although at first sight perhaps biologically unattractive, such a hyperbolic response function may result from an intrinsically first order system, when there is heterogeneity. Suppose IgG production and removal are distributed among several locations, all with different properties (different time constants and amplitudes). Sufficient heterogeneity among these sites could then result in a varying contribution of any given subsystem with time, with fast systems providing the bulk of the IgG in the initial phase of the response and ' recruiting' a smaller fraction of slower systems in the tail of the response.

Our analysis indicates that the IgG-PT response to an infection with *B. pertussis* shows a typical pattern, with a rapid transient increase over a few days to weeks, followed by slow decay extending over several years. This response can be described with a mathematical model; there appears to be considerable variation among responses from individual patients. The remarkably large variation in responses cannot be neglected when these are employed to estimate the time since infection from a given IgG-titre in a randomly chosen subject.

Appendix

Calculation of times since infection

From the above analysis of the longitudinal study we have a function describing the IgG-PT level against time from infection (first diagnosis)

$$y = f(x; \boldsymbol{\theta})$$

in which the parameter vector $\boldsymbol{\theta}$ is stochastic, describing the variation in responses among patients. Suppose we are able to invert this function, i.e. given $\boldsymbol{\theta}$ and y (IgG-PT level) we can find a corresponding time since infection

$$x = f^{-1}(y; \boldsymbol{\theta}) = h(y; \boldsymbol{\theta})$$

We are only interested in the decreasing part of this function, leaving a monotonically descending function of titre with time from infection.

If times to infection ξ are distributed as $g(\xi; \boldsymbol{\lambda})$ the response of patient with response function parameter vector $\boldsymbol{\theta}$ would lead to a titre density

$$\gamma(y; \boldsymbol{\theta}, \boldsymbol{\lambda}) = \begin{cases} 0 & \text{if } y > y_{\max}(\boldsymbol{\theta}) \\ -g\big(h(y; \boldsymbol{\theta}); \boldsymbol{\lambda}\big) h'(y; \boldsymbol{\theta}) & \text{otherwise} \end{cases}$$

$y_{\max}(\boldsymbol{\theta})$ is the maximum IgG-PT level reached by this patient (having parameter vector $\boldsymbol{\theta}$). This is different for each individual patient.

If, further, any patient's response is equally likely, the probability density of titres is

$$\gamma(y; \boldsymbol{\lambda}) = \int \gamma(y; \boldsymbol{\theta}, \boldsymbol{\lambda}) \mathrm{d}\boldsymbol{\theta}$$

Integration is done over all possible parameter combinations and may be based on the posterior Markov chain.

If we now have a cross-sectional sample of IgG-PT titres: $\{Y_{1,}, \cdots, Y_M\}$ the likelihood

$$\ell(\boldsymbol{\lambda}) = \prod_{i=1}^{M} \gamma(Y_i; \boldsymbol{\lambda})$$

allows estimation of the parameter vector $\boldsymbol{\lambda}$ of the distribution of times to infection $g(\xi; \boldsymbol{\lambda})$.

References

1. de Melker HE, Versteegh FGA, Conyn-van Spaendonck MAE, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. J Clin Microbiol 2000; 38: 800–6.

2. Guris D, Strebel PM, Bardenheier B, et al. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990–1996. Clin Infect Dis 1999; 28: 1230–7.

3. Tomoda T, Ogura H, Kurashige T. Immune responses to *Bordetella pertussis* infection and vaccination. J Infect Dis 1991; 163: 559–63.

4. 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: 908–10.

5. 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 Infect Dis 1995; 27: 273–7.

6. Giammanco A, Taormina S, Genovese M, Mangiaracina G, Giammanco G, Chiarini A. Serological responses to infection with *B. pertussis*. Dev Biol Standard 1997; 89: 213–20.

7. Di Tommaso A, Bartalini M, Peppoloni S, Podda A, Rappuoli R, De Magistris MT. Acellular pertussis vaccines containing genetically detoxified pertussis toxin induce long-lasting humoral and cellular responses in adults. Vaccine 1997; 15: 1218–24.

8. Giuliano M, Mastrantonio P, Giammanco A, et al. Antibody kinetics and long-term sero-prevalence in the Italian clinical trial of acellular pertussis vaccines. Dev Biol Standard 1997; 89: 275–8.

9. Giuliano M, Mastrantonio P, Giammanco A, Piscitelli A, Salmaso S, Wassilak SGF. Antibody responses and persistence in the two years after immunization with two acellular vaccines and one whole-cell vaccine against pertussis. J Pediatr 1998; 132: 983–8.

10. Bell F, Martin A, Blondeau C, Thronton C, Chaplais J, Finn A. Combined diphtheria, tetanus, pertussis and *Haemophilus influenzae* type b vaccines for primary immunisation. Arch Dis Child 1996; 75: 298–303.

11. de Melker HE, Schellekens JFP, Neppelenbroek SE, Mooi FR, Rümke HC, Conyn-van Spaendonck MAE. Reemergence of pertussis in the highly vaccinated population of the Netherlands : observations on surveillance data. Emerg Infect Dis 2000; 6: 348–57.

12. Versteegh FGA, Schellekens JFP, Nagelkerke AF, Roord JJ. Laboratory-confirmed reinfections with *Bordetella pertussis*. Acta Paediatr 2002; 91: 95–7.

13. Nagel J, de Graaf S, Schijf-Evers D. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LPF antibody levels. Dev Biol Standard 1985; 61: 325–30.

14. de Melker HE. Seroepidemiology of diphtheria, tetanus, poliomyelitis and pertussis. PhD thesis,

Wageningen University, 1999.

15. Gilks WR, Richardson S, Spiegelhalter DJ, editors. Markov Chain Monte Carlo in practice. London: Chapman and Hall, 1996.

16. Teunis PFM, Evers EC, Slob W. Analysis of variable fractions resulting from microbial counts. Quant Microbiol 1999; 1: 63–88.

17. van Herck K, Beutels P, van Damme P, et al. Mathematical models for assessment of long-term persistence of antibodies after vaccination with two inactivated hepatitis A vaccines. J Med Virol 2000; 60: 1–7.

18. Huang LM, Lee CY, Lin TY, Chen JM, Lee PI, Hsu CY. Responses to primary and a booster dose of acellular, component and whole-cell pertussis vaccines initiated at 2 months of age. Vaccine 1996; 14: 916–22.

19. Van Savage J, Decker MD, Edwards KM, Sell SH, Karzon DT. Natural history of pertussis antibody in the infant and effect on vaccine response [see comments]. J Infect Dis 1990; 161: 487–92. J Infect Dis 1990; 161: 473–9.

20. Hall E, Parton R, Wardlaw AC. Time-course of infection and responses in a coughing rat model of pertussis. J Med Microbiol 199; 48: 95–8.

21. Gay NJ. Analysis of serological surveys using mixture models: application to a survey of parvovirus B19. Stat Med 1996; 15: 1567–73.

22. Auranen K, Eichner M, Kayhty H, Takala AK, Arjas E. A hierarchical Bayesian model to predict the duration of immunity to *Haemophilus influenzae* type b. Biometrics 1999; 55: 1306–13.

23. Auranen K. Back-calculating the age-specific incidence of recurrent subclinical *Haemophilus influenzae* type b infection. Stat Med 2000; 19: 281–96.

24. van Herck K, van Damme P. Inactivated hepatitis a vaccine-induced antibodies : follow-up and estimates of long-term persistence. J Med Virol 2001; 63: 1–7.

25. Tiru M, Hallander HO, Gustafsson L, Storsaeter J, Olin P. Diphtheria antitoxin response to DTP vaccines used in Swedish pertussis vaccine trials, persistence and projection for timing of booster. Vaccine 2000; 181: 2295–306.

Chapter 5

Age-specific long-term course of IgG antibodies to pertussis toxin after symptomatic infection with *Bordetella pertussis*

F.G.A. Versteegh [1], P.L.J.M. Mertens [2], H.E. de Melker [3],

J.J. Roord [4], J.F.P. Schellekens [3], P.F.M. Teunis [3]

1: Groene Hart Hospital, dept. of Pediatrics, Gouda, the Netherlands

2: Municipal Health Department, Rotterdam, the Netherlands

3: National Institute of Public Health and the Environment, Diagnostic Laboratory of Infectious Diseases and Perinatal Screening, Bilthoven, the Netherlands

4: Free University Medical Centre, dept. of Pediatrics, Amsterdam, the Netherlands

Summary

To investigate the possible dependence on age of the rate of decline of IgG antibodies to pertussis toxin (IgG-PT) after natural infection with *Bordetella pertussis* we measured IgG-PT in follow-up sera of 121 patients (age 0-94 years) obtained after 123 episodes *B.pertussis* infection. For analysis we applied a dynamic model for the inactivation of *B.pertussis* by the immune system. There were no significant differences in rise, peak and decline of IgG-PT between different age groups, although there was a tendency for a more rapid increase, a higher peak and a faster decline with increasing age. The IgG-PT cut-off of 100 U/ml for serodiagnosis of pertussis appeared valid in all age groups. A decline of IgG-PT to < 10 U/ml was associated with increased risk of re-infection with *B.pertussis*.

Introduction

Neither vaccination against pertussis nor natural infection with *Bordetella pertussis* affords lifelong immunity (1-12). However, the rate of waning of immunity and possible differences after both vaccination and natural infection are difficult to establish, because no method exists to unequivocally measure (non)-susceptibility for pertussis for each individual. A simple single correlate of protection has not been found; both humoral and cellular immunity play a role (13,14). However, a relationship between protection and levels of IgG antibodies against the virulence factors pertussis toxin, pertactin and fimbriae has been demonstrated (2,13,15-17). In accordance with the concept of waning immunity, it has been observed that elevated levels of these IgG antibodies, whether induced by vaccination or natural infection, quite rapidly decrease again (9,13,18,19). However, the rate of decline and its possible age dependency have not been established. Also the question of whether or not after infection IgG-antibodies to PT (IgG-PT) eventually persist at some level or completely disappear, remains unanswered.

Of the mentioned virulence factors, pertussis toxin is of special interest: when used as a single component in an acellular vaccine, it induces considerable protection and is the only virulence factor which is present in each of the newly developed acellular pertussis vaccines (20). Furthermore, IgG-PT is an important parameter for serodiagnosis of pertussis, because cross-reactivity of pertussis toxin with antibodies induced by heterologous proteins has never been established and most individuals produce high levels of IgG-PT in response to infection with *B.pertussis* (19,21,22).

In our first study of the longitudinal course of IgG-PT after typical symptomatic infection with *B.pertussis*, practically all 57 participating pertussis patients were young children and an established model for assessing the rate of decline of IgG-PT antibodies was lacking (19). For assessment of the possible age dependence of the rate of IgG-PT decline after natural

infection, we extended our studies to a larger group of pertussis patients of various ages for whom follow-up sera were available and to a group of elderly adult patients with pertussis for whom a follow-up serum sample had been drawn 1 year after infection with *B.pertussis* (23). For analysis we applied an adapted version of a recently described dynamic model for the inactivation of *Bordetella pertussis* by the immune system (24).

Materials and Methods

Patients and sera

The first group of pertussis patients studied (A) consisted of 87 subjects (80 children and adolescents between 0 and 18 years and seven adults between 30 and 42 years), who suffered from clinically typical whooping cough (paroxysmal coughing for ≥ 2 weeks) between 1989 and 1999, confirmed by positive IgG-PT serology (for criteria see below). These patients were from the paediatric practice of one of the authors (F.G.A.V.) in Gouda, The Netherlands. After the pertussis episode (diagnosed by F.G.A.V.), they remained as a patient in that practice, often because of asthma or other respiratory problems. At the time of the pertussis episode, these patients or their parents gave informed consent to use future sera, which might be obtained for various reasons at irregular control visits or at consultations with new complaints, for measurement of IgG antibodies to pertussis toxin. The adults in this group were parents of participating children who had pertussis at the same time as their child and who had volunteered to participate in this study. Only the patients for whom at least one follow-up serum, obtained > 6 months after onset of disease, was available, were included in this analysis. The Medical Ethical committee of the Groene Hart Hospital approved the study. The second group (B) of pertussis patients studied consisted of 34 people from a convent in the southern part of The Netherlands, 33 nuns (age between 59 and 94 years) and 1 employee (aged 26 years). All of them had clinically typical pertussis during an outbreak of pertussis in this convent in 1992, which was confirmed by positive IgG-PT serology (for criteria see below) (for a detailed description of this outbreak see ref. 23). Approximately 1 year after the outbreak one follow up serum sample had been obtained from all patients. All patients gave informed consent.

Laboratory Evaluation

In both patient groups the clinical diagnosis of pertussis had been confirmed by finding a significant (i.e. ≥ fourfold) increase of IgG-PT in paired sera to a level of at least 20 U/ml in sera obtained within 3 months after onset of disease, or by the finding of a high IgG-PT concentration in a single serum sample, i.e. above a defined diagnostic cut-off of 100 U/ml as measured in an in-house IgG-PT ELISA of the National Institute of Public Health and the Environment, Bilthoven, The Netherlands (19). Previously, it was shown that the sensitivity of

these criteria for diagnosis of actual or very recent infection with *B.pertussis* was 90% in paired sera of two groups of patients with culture- or PCR-confirmed pertussis, studied several years apart (respectively n=89 and n=56) (19,25). The specificity of these criteria in paired sera of control-patients with respiratory infection of other etiology (n = 58) was 96% (25). The specificity of the ≥ 100 U/ml criterion for single sera was 99% when assessed in population sera (n=7756) and was independent of age (19). This ELISA, in which purified pertussis toxin is coated after pre-coating with fetuine, was developed and described at the start of the 1980s (26) and has been in use since then for serodiagnosis of pertussis for the whole country. For long-term consistency of potency expression in U/ml a serially diluted nationally standardized reference serum is applied on each ELISA plate. Routinely, two dilutions (1:100 and 1:400) of patient-sera are used and depending on the magnitude of optical densities (OD) of those dilutions, the OD of one of those is used to calculate the IgG-PT concentration of the patient-serum relative to the defined potency of the reference serum. Although the minimal quantitation in this assay is 1 U/ml, the coefficient of variation, being ~ 20% in the 5-500 U/ml range, is >30% in sera with values < 5 U/ml (27). Therefore, the detection-level of the assay is considered to be 5 U/ml. Due to the limited number of dilutions of patient sera used in the assay there is an upper limit of quantitative differentiation of 500 U/ml. However, the sera of patients from group B with IgG-PT ≥ 500 U/ml were subsequently retested in higher dilutions (1:800, 1:1600 and 1:3200) to obtain an exact value.

Recently, the IgG-PT ELISA used here (19,26) has been compared to an internationally standardized IgG-PT ELISA which was recommended by the United States Food and Drug Administration (FDA) for use in clinical trials of pertussis vaccines and in which the "lot 3 pertussis serum" of the FDA, with predefined content of IgG-PT in IU/ml, is used as reference serum (27-29). It was shown by Giammanco et al that there was a good correlation between both assays and that the relation between IU/ml in the internationally standardized ELISA and U/ml in our ELISA as calculated through linear regression of log-transformed concentrations is as follows: 5 IU/ml (detection level) = 6.5 U/ml and 125 IU/ml = 100 U/ml (diagnostic cut-off) (29).

Data analysis
IgG-PT responses show considerable variation among individual patients, as is clear in Figure 1. Therefore we analyse these responses with a hierarchical model allowing for variations between individual patients. For any individual patient the amount of information is limited: only a few measurements at best. For that reason we have described longitudinal responses with a model of the interaction between bacteria and the immune system. The model assumes that during infection bacteria are growing exponentially in the host. At the same time, bacteria are inactivated (killed) by antibodies (or by some action associated with the antibody response) according to a mass-action mechanism: inactivation depends on

the product of the concentrations of bacteria and antibodies (or antibody producing cells). Conversely, antibody production is controlled by the probability of antibodies (or antibody producing cells) encountering bacteria (or being presented with antigens derived from these pathogens). Antibody removal is considered an autonomous first order process. This is the simplest possible model for the interaction between host and pathogen and it is well known in population biology as a Lotka-Volterra model (30). The model can be formulated as a system of ordinary differential equations (see Appendix).
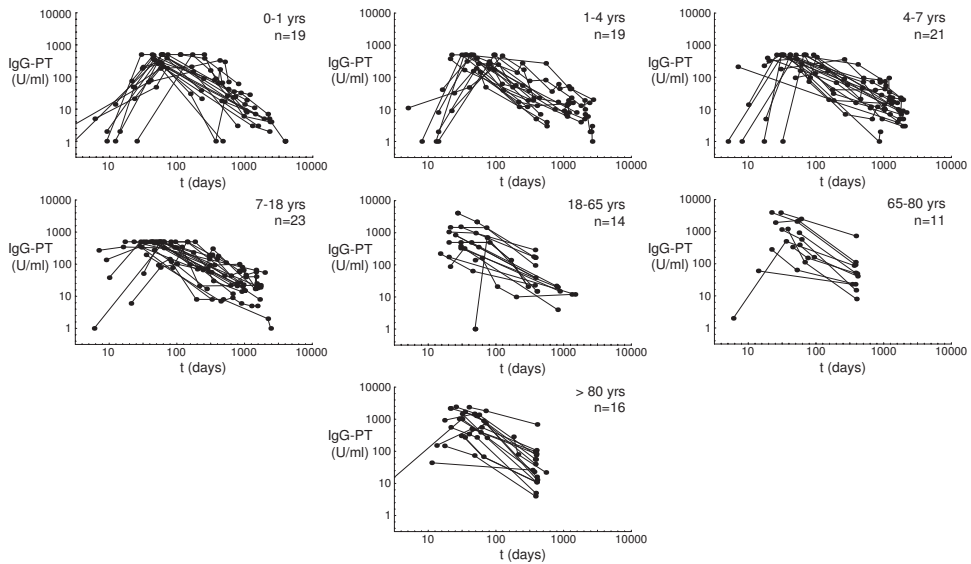
Fig. 1. Measured IgG-PT levels against time from infection [$\log_{10}$ (IgG-PT) against $\log_{10}$ (time)]. Different age groups are in separate panels; data from individual patients are connected.

This set of equations can be solved numerically and the solution is used for fitting to the patient data. Rather than fitting the model to data from any patient at any sampling time, individual records are fitted separately producing a set of parameters for each of the patients. We further use population distributions to describe the variation of these parameters among all the patients in an age group thereby generalizing the set of responses from individual patients to the age group level. Sampling from these population distributions then allows us to construct generalized immune responses typical for the whole age group instead of individual patients in that group. All our conclusions are based on these predicted responses. The model is fitted by means of a Markov Chain Monte Carlo (MCMC) algorithm (31) that allows efficient sampling of the parameter space for such complex multilevel problems. Some details are given in the appendix.

Results

Descriptive analysis

The age distribution of the 87 patients of group A is given in Figure 2. Pertussis-vaccination in The Netherlands at the time of the study consisted of 4 immunisations in the first year of life, with a nationally produced whole cell pertussis vaccine. Fifteen of the 19 patients < 1 year were not vaccinated at onset of pertussis or were incompletely vaccinated (fewer than three immunizations). In only one of those 15 was it documented that the patient had received immunisations against *B.pertussis* after the pertussis episode (note: at the time of the study it was normal practice to abandon [further] vaccination against *B.pertussis* in infants who had pertussis). In the other 68 patients five were not vaccinated. For two subjects the vaccination status was unknown (aged 30.2 and 35.6 years).
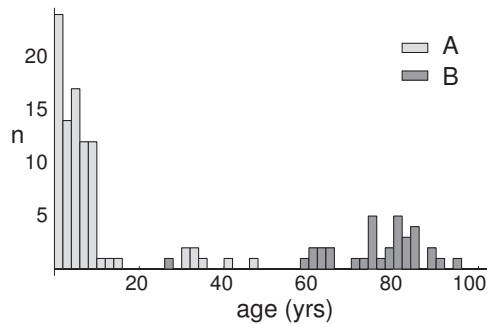

Fig. 2. Age distribution of pertussis cases from the two studies (note that these are 2-year categories, different from the age categories used for analysis). Patients <18 years are from group A, those >65 years from group B. Adults (18–65 years) are either parents of children in group A, or from group B.

In three of the 87 patients in group A during serological follow-up after pertussis a second infection with *B.pertussis* was serologically documented, respectively 3.4, 5.8 and 6.6 years after the first (see below for details). In those three patients the follow-up of the first infection with *B.pertussis* was considered to have ended with the last serum obtained before onset of the second infection with *B.pertussis*. The serological follow-up of the second episode in two of these three patients was > 6 months and inclusion of the follow-up of these two second episodes therefore resulted in follow-up in group A of 89 episodes of infection with *B.pertussis* in 87 patients. From the 89 follow-up periods in group A, 403 sera were available. The distribution of those sera over time since onset of pertussis is shown in Table 1. The mean number of sera for each follow-up period was 4.5 (range of 2-12). As shown in Figure 3 the time between onset of infection with *B.pertussis* and obtaining the last follow up serum sample ranged from 6 months to 10.7 years with a median of 3.1 years.

Table 1. *Percentages of pertussis episodes with indicated category of IgG-PT serum-concentration within the indicated time category*

	Time after onset of disease					
	0–6 mo.	>6–12 mo.	>1–2 yr	>2–4 yr	>4–6 yr	>6–11 yr
Group A						
No. episodes (no. sera) …	89 (180)	39 (49)	32 (44)	48 (75)	30 (40)	8 (15)
IgG-PT concentration (U/ml)						
>400	57	5	0	0	0	0
>100 to ⩽400	43	18	19	0	0	0
>20 to ⩽100	0	51	41	42	27	0
>5 to ⩽20	0	26	28	48	46	38
⩽5	0	0	12	10	27	62
Group B						
No. episodes (no. sera) …	34 (64)	6 (6)	29 (29)			
IgG-PT concentration (U/ml)						
>400	74	0	7			
>100 to ⩽400	21	17	14			
>20 to ⩽100	5	83	48			
>5 to ⩽20	0	0	24			
⩽5	0	0	7			

Separate data for group A (89 episodes in 87 patients) and group B (34 episodes in 34 patients). Times in months (mo.) or years (yr). In case of availability of multiple sera from one episode within one time category, the serum with the highest value has been used. Apart from the numbers of episodes with at least one serum available in the indicated time category (no. episodes) also the total numbers of sera available from those episodes in that time category (no. sera) is indicated.

The age distribution of the 34 patients of group B is also given in Figure 2. Since the national vaccination program in The Netherlands against pertussis started in 1952, the 33 patients of group B who were born before 1952 (i.e. all the nuns) had not been vaccinated.
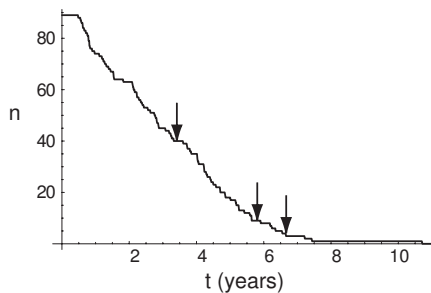
Fig. 3. Number of times patients of group A have been in the study; numbers of patients as a function of the time between onset of disease and last serum sample. The arrows denote time points of confirmed re-infections (one at each time point).

The one patient born after 1952 (the employee) had been completely vaccinated. From the 34 pertussis-episodes of the 34 patients of group B 99 sera were available, with 64 sera in the first 6 months after onset, six sera from six patients in the second half year and 29 sera from 29 patients in the second year (Table 1). The mean number of sera per patient/episode was 2.97 (range of 2 to 3). The time between onset of pertussis and obtaining the last follow up serum sample ranged from a minimum of 11.1 months to a maximum 17.6 months with a median

of 12.6 months.

Categorization of IgG-PT values (< 5, 5-20, 20-100, 100-400 and > 400 U/ml) and of time elapsed since onset of pertussis (0-6 months, 6-12 months, 1-2 years, 2-4 yrs, 4-6 yrs, 6-11 yrs) yielded the results shown in Table 1. All 89 episodes followed in group A were associated with an IgG-PT of > 100 U/ml at some time-point in the first six months after pertussis, while in the second half year and in the second year after pertussis the IgG-PT in >70% of cases had fallen below the level of 100 U/ml and in some had already fallen below the detection-level of 5 U/ml. Six to 11 years after pertussis the IgG-PT had declined to < 5 U/ml in five out of eight cases (62%); in the other three IgG-PT had declined to a level between 5 and 20 U/ml (Table 1).

Of the patients of group B 95% had an IgG-PT value of > 100 U/ml at some point in the first 6 months after pertussis (Table 1). In the two patients with IgG-PT < 100 U/ml in the first six months, a more than fourfold increase of IgG-PT in paired sera to a value between 50 and 100 U/ml had been found during the pertussis episode. In the second half year and in the second year after onset of pertussis > 70% of the patients of whom sera of that period were available had an IgG-PT < 100 U/ml and in one patient a value < 5 U/ml was found in the second year after infection.

The distribution of IgG-PT categories in groups A and B in the first 6 months after infection (for each individual the highest value in that period is taken) and in the second year after infection (also highest value) are remarkably similar suggesting that the rate of decline over time in both groups was similar (Table 1).

Re-infections

In the three patients in group A with re-infection after 3.4, 5.8 and 6.6 years, the maximum IgG-PT titre in the first months after the first typically symptomatic infection was ≥500, ≥500 and 248 U/ml respectively, the high titres had declined in sera obtained after 2.8, 2.9 and 3.1 years respectively (i.e. the last serum before onset of the second episode of infection), to 10, 8 and 6 U/ml respectively. The maximum IgG-PT titre reached in the first months after onset of the second episode was 492, 156 and 270 U/ml respectively. The patient with re-infection after 3.4 years (first infection at age 4.2 years) had had a very mild cough, lasting 1 week, 4 weeks before sampling of the serum in which a high IgG-PT, diagnostic of very recent infection, was found. At the same time a sibling had serological confirmed pertussis. The patient with re-infection after 5.8 years (first infection at age 0.4 years) was discovered when blood was sampled at that time for other reasons of; the parents recalled no recent coughing illness. The patient with re-infection after 6.6 years (first infection at age 2.7 years) suffered from mild coughing during 3 weeks; besides the finding of a high IgG-PT concentration diagnostic for very recent infection, this patient was also pertussis-PCR positive.

In Figure 3 the times (after first infection) of occurrence of the three re-infections have been

indicated. It can be seen that the one re-infection after 3.4 years was among 40 patients with a follow up of 3.4 years or more (2.5%), the one re-infection after 5.8 years was among 9 patients with a follow up of 5.8 years or more (11%) and the one re-infection after 6.6 years was among 4 patients with a follow up of 6.6 years or more (25%).

Analysis in dynamic model

For analysis in the dynamic model, the patients of group A and B were taken together and divided into seven age groups: (a)<1 year (n=19), (b)1–4 years (n=19), (c)4-7 years (n=21), (d)7-18 years (n=23), (e)18-65 years (n=14, seven from group A, seven from group B), (f)65-80 years (n=11) and (g) ≥ 80 years (n=16).

Table 2. *Predicted times (in months post onset of symptoms), to reach peak levels of the IgG-PT response, and (during the declining phase of the IgG-PT response) to reach 100 U/ml and times to reach 5 U/ml: $Q_{0.05-0.50-0.95}$=5th, 50th (median) and 95th percentiles*

Age (years)	Time to peak			Time to 100 U/ml			Time to 5 U/ml		
	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$
0–1	0·55	1·18	2·94	1·34	8·28	38·90	15·11	36·48	104·36
1–4	0·47	1·45	2·72	1·48	5·30	97·83	4·63	26·27	257·39
4–7	0·41	0·96	1·86	1·80	14·06	34·40	21·74	62·06	159·55
7–18	0·38	0·77	1·63	3·12	12·25	29·09	20·11	47·93	113·90
18–65	0·09	0·31	1·34	2·29	8·73	25·23	4·56	23·16	100·32
65–80	0·14	0·45	1·20	2·73	10·63	24·61	8·63	25·48	75·67
≥80	0·15	0·40	1·52	2·08	6·61	19·44	3·75	17·13	68·55

Figure 1 shows the individual responses of IgG-PT to infection with *B. pertussis* in these different age groups. In groups (a)-(d) and part of group (e) (i.e. the patients in this age group derived from group A) the IgG-PT concentrations are censored at an upper level of 500 U/ml (see Laboratory evaluation section above). In part of group (e) (those derived from group B) and in groups (f)-(g) the IgG-PT concentrations are given. The regression model allows for censoring of IgG-PT data from group A (as reported previously (24)), making groups A and B comparable. Note that in Figure 1 both IgG-PT concentrations and time elapsed since onset of pertussis are log-transformed, which is common for antibody concentrations but is unusual for time. However, in this manner both the rapidly increasing as well as the slowly decreasing phase of the immunoresponse can be shown in a single graph.

In Figure 4 the estimated variation in individual IgG-PT responses in the different age groups as calculated in the dynamic model is shown as the median (50th percentile) and the 5th and 95th percentiles of simulated responses for each age group. In all groups there is considerable variation. The older groups (e)-(g) tend to have a higher median peak response, also with a wide range. The time to reach peak levels seems inversely correlated with age (Table 2). Group (b) (1-4 years) seems to have the lowest predicted peak level (Table 3) and the longest time to reach peak levels (Table 2). However, the response range in the 1-4 year age group was

wide and overlapped the corresponding ranges in the other age groups. The median time-periods in the different age groups for IgG-PT to reach the peak level and to decline to levels of respectively < 100 U/ml (the diagnostic cut-off) and < 5 U/ml (detection level) are given in Table 2. These data also indicate that there might be an age dependent tendency for older people to have a faster decline. Eventually, the median time for IgG-PT to decrease below the detection-level of 5 U/ml varied per age group from 1.4 to 5.2 years, with wide inter-individual variations (Table 2).
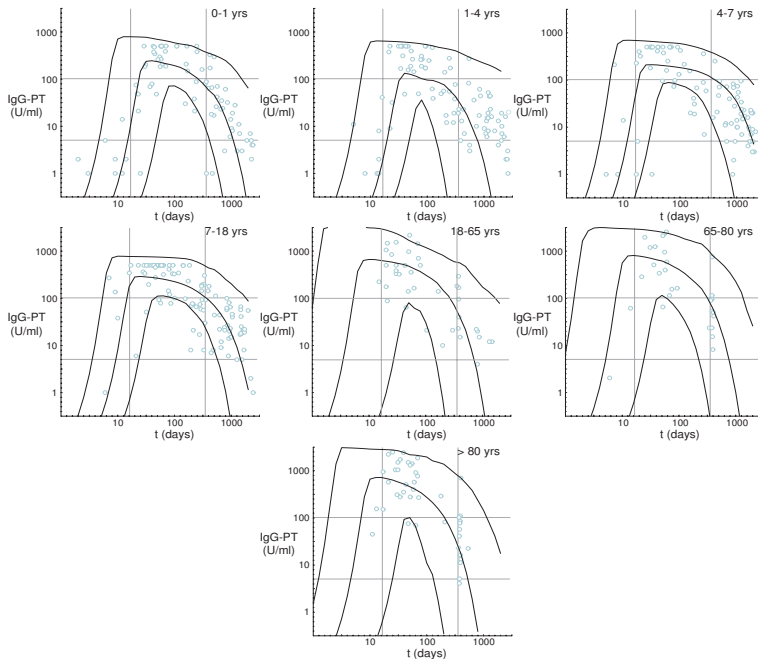

Fig. 4. Predicted IgG-PT antibody responses to infection in each of the age groups, on a $\log_{10}$ (IgG-PT) $-\log_{10}$ (time) scale to show both initial rise and final decline of the responses. Intervals show (95 %) range of variation in response among individual patients. Circles indicate observed IgG-PT titres. Also indicated are 3 weeks and 1 year past onset of symptoms (vertical lines), and IgG levels (horizontal lines) of 5 U/ml (detection level), and 100 U/ml (diagnostic cut-off).

The estimated variation in the older groups (e)-(g) is higher than in groups (a)-(d). While it cannot be excluded that this is also -in part- caused by the influence of censoring, it should also be noted that the records of older patients (of group B) consist of fewer measurements (two or three) than those of the juvenile patients (of group A). We checked the influence of censoring by recalculation after artificially censoring the data from group B, and found similar results, confirming that correction for censoring of the data from group A in the dynamic model was appropriate (data not shown).

In Figure 5 the predicted age profile of IgG-PT responses at different time-points after onset of pertussis is given (10, 20, 50, 100, 200, 365 and 730 days). At 10 and 20 days there is large variation in response in all age groups, possibly partly due to large variations in the rapidity of
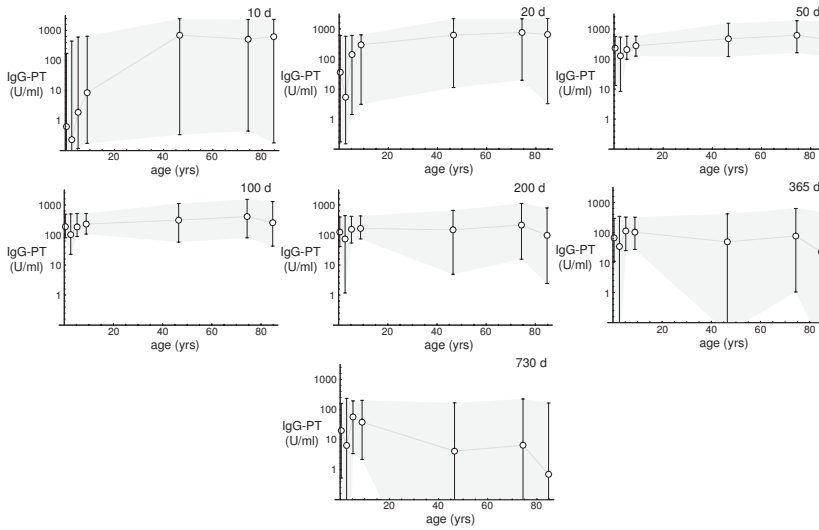
Fig. 5. Age profiles of ($\log_{10}$) IgG-PT responses: predicted antibody levels against age (category) at various times from infection. As in Figure 4 the intervals show (95%) range of variation in response among individual patients.

the increasing phase of the IgG-PT response to infection and possibly partly due to inaccurate reports of the first day of the disease.

However, at both time-points the IgG-PT concentrations in the three adult subgroups tended to be higher, suggesting that the IgG-PT response to infection in adults may be somewhat more rapid. At 50 days and at 100 days all predicted median IgG-PT levels are > 100 U/ml and the variation of responses is small, without substantial differences among age groups, although peak responses in the oldest two subgroups appeared to be somewhat higher. At 200 days antibody decay has started in all age groups. In the older patients (65 to <80 and $\geq$80 years) there seemed to be a tendency towards a more rapid decline of IgG-PT after 365 and 730 days than in younger patients.

Table 3. *Predicted peak levels (U/ml) of the IgG-PT response:* $Q_{0.05-0.50-0.95}$ = *5th, 50th (median) and 95th percentiles*

Age (years)	Peak titre (U/ml)		
	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$
0–1	85·9	244·3	624·7
1–4	46·2	136·2	575·1
4–7	100·3	213·0	621·6
7–18	124·3	303·0	653·7
18–65	138·7	695·2	2631·6
65–80	188·2	792·6	2384·2
>80	162·1	720·5	2503·3

The second youngest age group (1-4 years) appears to show a response which is slightly different from the other age groups (slower increase, lower magnitude). As already stated, data in this group show markedly high dispersion, as may also be appreciated by comparing error bars (illustrating variation) in Figure 5 (see also Fig.4 and Tables 2 and 3).

Discussion

This study in 121 pertussis patients aged 0-94 years shows that elevated levels of IgG-PT induced by infection with *B.pertussis* consistently decline again to below detection levels within several years, unless this course is interrupted by re-infection with *B.pertussis*, which was observed in three cases. In each of the different age groups there was wide variation in rapidity, intensity and rate of decay of IgG-PT and between groups there was a wide overlap of the ranges of these variables. Although the pattern of IgG-PT response is not significant age dependant, there was a tendency for the IgG-PT response after infection to be faster and stronger and the decay to be more rapid with increasing age. The pattern in the 1-4 y age group was different from all other age groups in that the IgG-PT response tended to be slower mounting and smaller in amplitude and in most cases decayed rapidly.

Our study is unique with respect to the high number of participating patients, the wide age range of the patients, the long follow up times in part of the patients and also the method of analysis using a hierarchical model for induction and decay of antibodies after infection. The few studies of the course of IgG-PT after infection with *B.pertussis* that have been published all show decline over time but assessment of possible differences in children and adults is lacking and follow up times are relatively short. In the studies of Hodder et al (9) and Heininger et al (32) the course of IgG-PT over a period of 20 months after pertussis in 48 adults aged >65 years (9) and over a period of 28 months in 11 adults (32) was remarkably similar to the pattern over the same time span in adults in our study. In those studies the internationally standardized IgG-PT ELISA, recommended by FDA, was used of which the relationship with our ELISA is known (see Materials and Methods section; (29)). For instance, the geometric mean IgG-PT titre of 11 adult pertussis patients was 242 IU/ml at 2 months after onset of infection and 45 IU/ml at 28 months after onset (32). However, in none of those patients IgG-PT had declined below detection levels, which may be related to the limited follow up time. Two other studies, one comprising both adults and children (2) and one comprising young children (33), also showed a significant decline of geometric mean IgG-PT titre 1 year after pertussis. The study with the longest follow up time was from Tomoda et al. who measured IgG-PT during an outbreak of pertussis in a semi-closed adult community and again 5 years later (34). He showed that in the large majority of the 21 pertussis-patients of that outbreak (37.5 ± 12.1 years) IgG-PT was > 50 U/ml several weeks after onset of pertussis; 5 years later the IgG-PT in all patients had declined to < 10 U/ml and in most to undetectable levels.

These findings support our conclusion that in the majority of cases or perhaps in all cases of pertussis, the subsequent decay of infection-induced IgG-PT does not level off well above detection thresholds but progresses to undetectability.

The relatively short persistence, in all age categories, of infection-induced peak levels of IgG-PT supports our previous finding that a diagnostic cut-off of 100 U/ml (equivalent to 125 IU/ml) for serodiagnosis of actual or very recent infection with *B.pertussis* is valid for all ages (19). Although vaccination can induce IgG-PT levels > 100 U/ml, interference with serodiagnosis of pertussis is minimal because IgG-PT induced by vaccination with whole cell pertussis vaccines or acellular pertussis vaccines also declines quite rapidly. Multiple studies show a decrease after primary and booster vaccination to very low or undetectable levels within 1 to 8 years (13, 35-38). For instance, Taranger et al (39) followed 813 children after pertussis vaccination with a monovalent pertussis toxoid vaccine. There was a strong IgG-PT response to a geometric mean of 143 IU/ml at 1-2 months after vaccination and a rapid decrease to a geometric mean of 8 IU/ml at 21-32 months after vaccination.

One explanation for our finding of a relatively slow and low IgG-PT response in the 1-4 years age group, the large majority of whom had been (recently) vaccinated, might be that children who suffer from *B.pertussis* infection shortly after vaccination are a separate category, i.e. are children with a relatively poor immunoresponse to pertussis antigens. Indeed, Taranger et al (39) showed that previously vaccinated children who within 33 months after completing vaccination developed pertussis upon exposure in their household had had a significantly lower IgG-PT response 1-2 months after the third vaccination with a monocomponent (PT) acellular vaccine (mean peak-response 79 U/ml) than children who did not develop pertussis upon household exposure within that time-frame (mean peak response after vaccination 212 U/ml).

The tendency of the IgG-PT response to be more rapid and strong with increasing age may indicate the involvement of specific memory immunity through encounters with *B.pertussis* antigens earlier in life. Also Granström et al (40) have shown that adults have a faster peak response than children after a natural infection. The intuition that such rapid and strong responses would persist longer evidently is not true. Perhaps the rapid decay of IgG-PT after a rapid and strong response may be explained by a faster eradication of the pathogen and shorter duration of antigenic stimulation of the immune system. The phenomenon as such has been noted before: Blennow & Granström (41) showed that children receiving a booster vaccination with an acellular vaccine containing pertussis toxin and filamentous haemagglutinin showed a more rapid decay of neutralizing antibodies to pertussis toxin in the Chinese hamster ovary cells assay than after the primary vaccination. This despite the fact that after the booster vaccination the median of the neutralizing antibodies titres was higher than after the primary vaccination.

The three re-infections documented in this study were in children whose IgG-PT at the time

of the second infection had declined to <10 U/ml. Although the number of re-infections was small, their timing and incidence was compatible with the statement that natural infection initially induces protection against re-infection but that susceptibility to (re-)infection re-emerges when IgG-PT concentrations have fallen to < 10 U/ml and that subsequently susceptibility for disease in association with infection increases over time. In a previous paper, we have described two other patients who had infection with *B.pertussis* 12 years after the first episode. In contrast to the three re-infections 3.4, 5.8 and 6.6 years after the first in this study, the re-infections after 12 years were associated with typical symptoms i.e. long-lasting paroxysmal cough (12).

In conclusion this study shows that the high IgG-PT concentrations induced by infection with *B.pertussis* consistently decline to low or undetectable levels within 5-6 years, this decline is associated with emergence of susceptibility for re-infection and disease. Although the pattern of decline is largely independent of age, there is a tendency for older people to have a more rapid increase, higher peak and faster decline. This study also shows that a diagnostic cut-off of 100 U/ml is not only valid in children (19) but is true for all ages.

Appendix

The predator-prey model of the interaction between the immune system and invading bacteria can be formulated as:

$$\begin{cases} x'(t) = ax(t) - bx(t)y(t) \\ y'(t) = -cy(t) + dx(t)y(t) \end{cases} \quad \begin{cases} x(0) = x_0 \\ y(0) = y_0 \end{cases}$$

(pathogen concentration $x(t)$; antibodies $y(t)$; pathogen growth rate a; antibody dependent pathogen inactivation b; antibody decay rate c; pathogen dependent antibody production rate d).

Numerical solutions to these equations (a simple analytical solution does not exist) can be fitted to the longitudinal antibody data, treating the unknown pathogen concentration as a nuisance variable.

Measurement errors were assumed lognormal: both data and model function were log-transformed (TBS: Transform Both Sides) so that we could use a normal likelihood function. This also allows treatment of censoring as previously reported (24)). Variation between individual patients is considerable, we therefore used a hierarchical Bayesian model. For statistical analysis, parameters were transformed:

$$\begin{aligned} u &= \sqrt{ac} & w &= \sqrt{bd} \\ v &= \sqrt{a/c} & z &= \sqrt{b/d} \end{aligned}$$

and these new parameters were log transformed to restrict them to positive values. Parameters u and v were assumed equal in all patients, w and z vary among individuals. Parameter estimation for the hierarchical model was performed using a MCMC method, employing the Metropolis-Hastings algorithm (31).

Posterior predictive intervals for the longitudinal response to pertussis were constructed by applying the longitudinal model to a parameter sample from the MCMC output (by addition of a subject with missing data only, for conveniently sampling from the population parameter distributions).

References

1. Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study. BMJ 1988;296:612-4

2. Long SS, Welkon CJ, Clark JL. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis 1990;161: 480-6

3. Wirsing von König CH, Postels-Multani S, Bock HL, Schmitt HJ. Pertussis in adults: frequency of transmission after household exposure. Lancet 1995;346:1326-9

4. Cromer BA, Goydos J, Hackell J, Mezzatesta J, Dekker C, Mortimer EA. Unrecognized pertussis infection in adolescents. Am J Dis Child 1993;147:575-7

5. Laing JS, Hay M. Whooping-cough: its prevalence and mortality in Aberdeen. Public Health 1902;14:584-98

6. Luttinger P. The epidemiology of pertussis. Am J Dis Child 1916;12:290-315

7. Long SS, Lischner HL, Deforest A, Clark JL. Serologic evidence of subclinical pertussis in immunized children. Pediatr Infect Dis J 1990;9:700-5

8. Lambert HJ. Epidemiology of a small pertussis outbreak in Kent Country, Michigan. Public Health Rep 1965;80:365-9

9. Hodder SL, Cherry JD, Mortimer Jr EA, Ford AB, Gornbein J, Papp K. Antibody responses to *Bordetella pertussis* antigens and clinical correlations in elderly community residents. Clin Infect Dis 2000;31:7-14

10. Deville JG, Cherry JD, Christenson PD et al. Frequency of unrecognized *Bordetella pertussis* infections in adults. Clin Infect Dis 1995;21:639-42

11. Strebel P, Nordin J, Edwards K et al. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995-1996. J Infect Dis. 2001;183:1353-9

12. Versteegh FGA, Schellekens JFP, Nagelkerke AF, Roord JJ. Laboratory-confirmed re-infections with *Bordetella pertussis*. Acta Paediatr 2002;91:95-7

13. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine 1998;16:1907-16

14. Hewlett EL, Halperin SA. Serological correlates of immunity to *Bordetella pertussis*. Vaccine 1998;16:1899-1900

15. Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. Vaccine 1998;16:1901-6

16. 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: 190-4

17. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Low levels of antipertussis antibodies plus lack of history of pertussis correlate with susceptibility after household exposure to *Bordetella pertussis*

Vaccine 2003;21:3542-9

18. Halperin SA, Scheifele D, Barreto L et al. Comparison of a fifth dose of a five-component acellular or a whole cell pertussis vaccine in children four to six years of age. Pediatr Infect Dis J 1999;18:772-9

19. Melker HE de, Versteegh FGA, Conyn-van Spaendonck MAE et al. Specificity and sensitivity of high levels of Immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. J Clin Microbiol 2000;38:800-6

20. Cherry JD, Olin P. The science and fiction of pertussis vaccines. Pediatr 1999;104:1381-4

21. Zee A van der, Agterberg C, Peeters M, Mooi F, Schellekens J. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis 1996;174:89-96

22. Müller F-MC, Hoppe JE, Wirsing von König CH. Laboratory diagnosis of pertussis: state of the art in 1997. J Clin Microbiol 1997;35:2435-43

23. Mertens PLJM, Stals FS, Schellekens JFP, Houben AW, Huisman J. An epidemic of pertussis among elderly people in a religious institution in the Netherlands. Eur J Clin Microbiol Infect Dis 1999;18:242-7

24. Teunis PFM, Heijden OG van der, Melker HE de, Schellekens JFP, Versteegh FGA, Kretzschmar M. Kinetics of IgG-antibody response to pertussis toxin after infection with *B. pertussis*. Epidemiol Infect 2002;129:479-89

25. Schellekens JFP, Boshuizen HC, Verbakel JMM et al. Serodiagnosis of Pertussis with Commercial ELISA's. ICAAC, Chicago USA, 22-25 september 2001, abstractbook page 163, abstract number D-1403

26. Nagel J, de Graaf S, Schijf-Evers D. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LPF antibody levels. Dev Biol Stand 1985; 61: 325-30

27. Lynn F, Reed GF, Meade BD. A comparison of enzyme immuno-assays used to measure serum antibodies to components of *Bordetella pertussis*. Dev Biol Stand 1997;89:197-204

28. Lynn F, Reed GF, Meade BD. Collaborative study for the evaluation of enzyme-linked immunosorbent assays used to measure human antibodies to *Bordetella pertussis* antigens. Clin Diagn Lab Immunol 1996;3:689-700

29. Giammanco A, Chiarini A, Maple PAC et al. European sero-epidemiology network: standardisation of the assay results for pertussis. Vaccine 2003;22:112-20

30. Edelstein-Keshet L. Mathematical Models in Biology. Birkhäuser Mathematics Series, New York, McGraw-Hill, 1988

31. Gilks WR, Richardson S, Spiegelhalter DJ eds., Markov chain Monte Carlo in practice, London, Chapman and Hall, 1996

32. Heininger U, Cherry JD, Stehr K. Serological response and antibody-titer decay in adults with pertussis. CID 2004; 38: 591-4

33. 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 Infect Dis. 1995;27:273-7

34. Tomoda T, Ogura H, Kurashige T. The longevity of the immune response to filamentous hemaglutinin and pertussis toxin in patients with pertussis in a semiclosed community. J Infect Dis 1992;166:908-10

35. Tomoda T, Ogura H, Kurashige T. Immune responses to *Bordetella pertussis* infection and vaccination. J Infect Dis 1991;163:559-63

36. Poovorawan Y, Theamboonlers A, Sanpavat S, Chumdermpadetsuk S, Safary A, Vandepapeliere P. Long-term antibody persistence after booster vaccination with combined tetravalent diphtheria tetanus, whole-cell *Bordetella pertussis* and hepatitis B vaccine in healthy infants. Ann Tropic Paediatr 1997;17:301-8

37. Giuliano M, Mastrantonio P, Giammanco A, Piscitelli A, Salmaso S Wassilak SGF. Antibody responses and persistence in the two years after immunization with two acellular vaccines and one whole-cell vaccine against pertussis. J Pediatr 1998;132:983-8

38. Tran Minh NN, He Q, Edelman K et al. Immune responses to pertussis antigens eight years after booster immunization with acellular vaccines in adults. Vaccine 2000;18:1971-4

39. Taranger J, Trollfors B, Lagergård T et al. Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. J Infect Dis 2000;181:1010-3

40. Granström M, Granström G, Gillenius P, Askelöf P. Neutralizing antibodies to pertussis toxin in whooping cough. J Infect Dis 1985;151:646-9

41. Blennow M, Granström M. Long term serologic follow-up after pertussis immunization. Pediatr Infect Dis J 1990;9:21-6

Chapter 6

The incidence of *Bordetella pertussis* infections estimated from a combination of serological surveys

H.E. de Melker [1], F.G.A. Versteegh [2], J.F.P. Schellekens [3], P.F.M. Teunis [4], M. Kretzschmar [1]

1: Department of Infectious Diseases Epidemiology, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

2: Department of Pediatrics, Groene Hart Hospital, Gouda, The Netherlands

3: Diagnostic Laboratory of Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

4: Computerization and Methodological Consultancy Unit, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Abstract

Objectives: *Bordetella pertussis* circulates even in highly vaccinated populations and there is a considerable amount of asymptomatic infection in adults. For designing more effective vaccination schedules it is important to quantify the age-dependent relation between the number of notified cases and the number of infections.

Methods: We used a statistical relationship between the time-since-infection and the IgG pertussis titers, derived from a longitudinal data set, to estimate time since infection for all individuals in a cross sectional population-based study (1995-1996) based on their titers. Age-specific incidence of infection with *B. pertussis* was calculated and compared with the age distribution of notified cases of pertussis in 1994-1996.

Results: Estimated incidence of infection was 6.6% per year for 3 to 79-years olds, annual incidence of notified cases 0.01%. Estimated age-specific incidence of infection was lowest for 3-4 year-olds and increased up to the age of 20-24 years. The number of notified cases was highest for 3 to 9 year olds.

Conclusions: *B. pertussis* infections occur frequently in the Dutch population, particularly in adults whose reported incidence is low. The age-distribution of infections differs notably from those of notified cases. Vaccination strategies should take into account age-specific circulation and contact patterns between age groups.

Introduction

Despite widespread vaccination, infection with *Bordetella* (*B.*) *pertussis* remains a cause of considerable morbidity even in countries with high vaccination coverage [1-5]. The continuing circulation of the pathogen is attributed to waning of vaccine-induced immunity, which leads to the occurrence of pertussis infection among previously vaccinated children, adolescents and adults [6-9]. In those groups, an infection with *B. pertussis* often goes undiagnosed, since the disease in many cases is atypical and mild and laboratory diagnosis is very insensitive, if only culture and/or PCR is available [8,10-13]. Like others, we have shown that availability of pertussis serology and particularly the definition of reliable criteria for positivity associated with high levels of IgG to pertussis toxin (IgG-PT) in a single serum sample (one-point serology) can greatly enhance the detection of pertussis infections [4,7,10].

Inclusion of positive one-point serology as laboratory confirmation for notification in the Netherlands has increased the notification rate considerably, especially among older children and adults [1]. However, the use of surveillance data of notified infections for understanding the epidemiology of pertussis remains limited by the lack of registration- or notification discipline. Furthermore, the fraction of infections diagnosed or reported is age-dependent

because the severity of the disease as well as the sensitivity of diagnostic methods is age-dependent [1]. Circulation of *B. pertussis* in vaccinated children, adolescents and adults plays an important role in the continuing transmission of the pathogen to infants too young to be vaccinated, in whom disease is most severe and possibly fatal [8,11-20].

To design better preventive measures, for example by determining the optimal ages for booster vaccinations, insight is needed into the age-specific incidence of all infections with *B. pertussis* as opposed to only those symptomatic infections that are diagnosed and reported. Information about the seroprevalence of IgG-PT antibodies in the general population in combination with knowledge about the rate of decline of IgG-PT antibody levels after infection with *B. pertussis* offers the opportunity to study the incidence of infection in various age-groups irrespective of clinical course, diagnosis and reporting frequency. Pertussis toxin is expressed only by *B. pertussis* and cross-reacting antigens have not been described [21,22].

Furthermore IgG-PT responses occur in most patients with *B. pertussis* infection and high levels persist only temporarily [10]. We estimated the incidence of *B. pertussis* infections in the population using a novel two-stage approach. A statistical description of the decline in antibody levels after infection as derived from a small scale longitudinal study [23] was combined with data about the age-specific distribution of IgG antibodies against pertussis toxin (IgG-PT) in sera derived from a large scale cross sectional study of the general population [10] to estimate the age-specific incidences of infection for the age range 3-79 years. These were compared to notification data of reported clinical cases of pertussis. Implications for vaccination-strategy are discussed.

Material and Methods

Collection of sera from population and patients

Detailed descriptions of the collection of sera from the general population in the Netherlands (n=7756) and of follow-up sera from patients with diagnosed clinical pertussis (n=85) have been published elsewhere [10, 23, 24]. In short, for the cross sectional study in the general population, eight municipalities were sampled with probabilities proportional to their population size within each of five geographical Dutch regions with similar population sizes. An age-stratified sample (classes 0, 1-4, 5-9, … 75-79 years] of 380 individuals was randomly selected from each municipality. Subjects were asked to give a blood sample. Samples were collected in the period from October 1995 to December 1996 and stored at –70 °C until use. The participation rate was 55 percent. Sufficient serum for pertussis serology was available for 7756 of 8359 participants.

In a longitudinal study [23], follow-up samples from 85 patients clinically diagnosed with pertussis (paroxysmal cough lasting more than two weeks) were obtained from one pediatric practice in the period 1989-2000. The follow-up period ranged from 6 months to 11 years

and the number of serial sera per patient ranged from 2 to 11. Eleven patients were less than 6 months of age at the time they contracted pertussis, 69 patients were between 6 months and 17 years of age and six patients were between 30 and 41 years old.

Notification data

Pertussis notification data for the period 1994 to 1996 were obtained from the Dutch Inspectorate of Health. The case definition included clinical symptoms and laboratory confirmation (or close contact with a person with laboratory-confirmed pertussis). The clinical symptoms in the case definition are a serious cough, lasting more than two weeks, coughing attacks, or coughing followed by vomiting in combination with at least one of the following symptoms/findings: apnea, cyanosis, characteristic cough with whooping, subconjunctival bleeding, or leucocytosis.

Laboratory confirmation was defined as either a positive culture of *B. pertussis* or *B. parapertussis*, or positive two-point serology. Two-point serology was considered positive if a 4-fold rise of IgG antibodies against pertussis toxin in paired sera was found. Only since April 1997, positivity of one point serology has been formally included in the case definition as being acceptable as laboratory confirmation of pertussis for notification. However, already in the years before 1997, a small fraction of the patients with positive one-point serology was reported [1].

Antibody assay

In the longitudinal study, patient sera had been submitted immediately after sampling and were assayed in the routine setting of the serology laboratory of our institute within 4 days after receipt. In the cross-sectional study, sera that had been collected in 1995/1996, were assayed in 1997/1998 in the same routine setting at a rate of approximately 200 sera per week. The IgG-PT was measured by ELISA as previously described [25]. In short, the procedure was as follows: purified PT (source: National Institute of Public Health and the Environment) was coated to 96-well ELISA plates after precoating with fetuin (50 mg/l in phosphate-buffered saline). Peroxidase labeled rabbit antihuman IgG was used as a conjugate and 3,3',5,5' tetramethylbenzidine (TMB) was used as the substrate. Negative, low-positive and medium-positive control serum with defined IgG-PT content was run on each plate. The sera were tested in duplicate in a 1:100 and 1:400 dilution against serial dilutions of a positive reference serum with a range of 1.6-100 "local" U/ml. The optical density (OD) of the 1:100 dilution was used for calculation of the IgG-PT concentration. When the OD of the 1:100 dilution of a serum was above the range that constituted the steep part of the dose response curve of the reference-serum, the OD of the 1:400 dilution was used. Due to the use of only two dilutions for the sera, the IgG-PT assay has an upper limit (500 U/ml) above which the values are not further differentiated. The lower detection limit of the assay is 5 U/ml. Results are expressed in "local U/ml".

ANALYSIS

In Teunis et al [23], a skewed hyperbolic function was used to describe the relationship between the log time since infection and the log antibody titer. This four-parameter function was fitted to the data for each individual patient from the longitudinal study of diagnosed clinical pertussis patients yielding a set of response curves with variation among individuals (Figure 1). On a linear time scale, the antibody response rises very fast (within a few days) and then declines very slowly over a period of several years. Therefore, the rising part of the response curve may safely be neglected and an inverse function can be determined on the basis of the long monotone declining part of the response. The inverse can be determined for each individual response curve separately. For every value of the log titer an average time since infection can be calculated (Figure 1).

We used those point-wise averages of the inverse response curves to estimate the times since last infection for the cross-sectional study population, assuming that the distribution of responses in the longitudinal study population is the same as that of the cross-sectional study population. Figure 2 shows the IgG-PT antibody distribution in the participants from the cross sectional population based study (adapted from [10]).
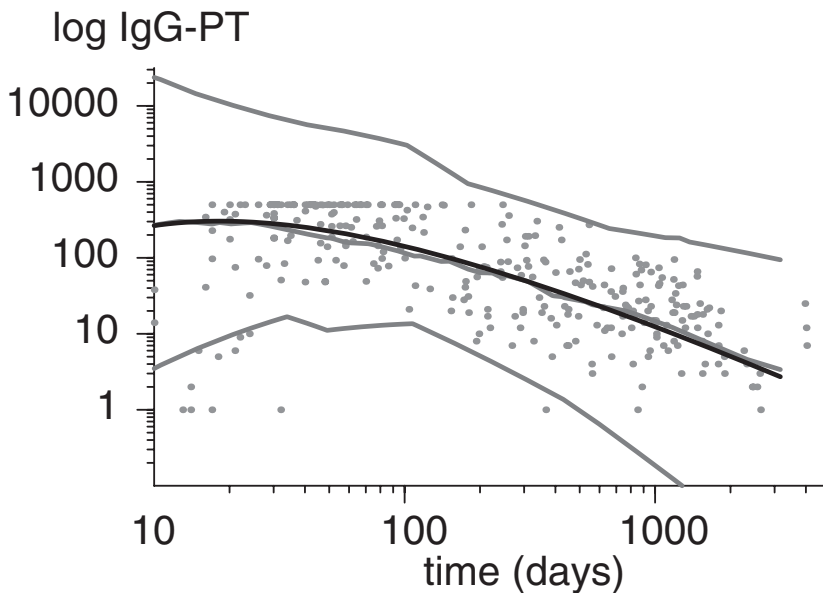

Fig 1: Measured IgG-PT concentration (titer) as a function of the time since last infection (for the study population in Teunis et al [23]). The black line shows the point wise average of individual estimates, the gray lines the point wise 2.5%, 50% (median) and 95% percentiles illustrating the magnitude of individual variation in responses. The dots are individual results.
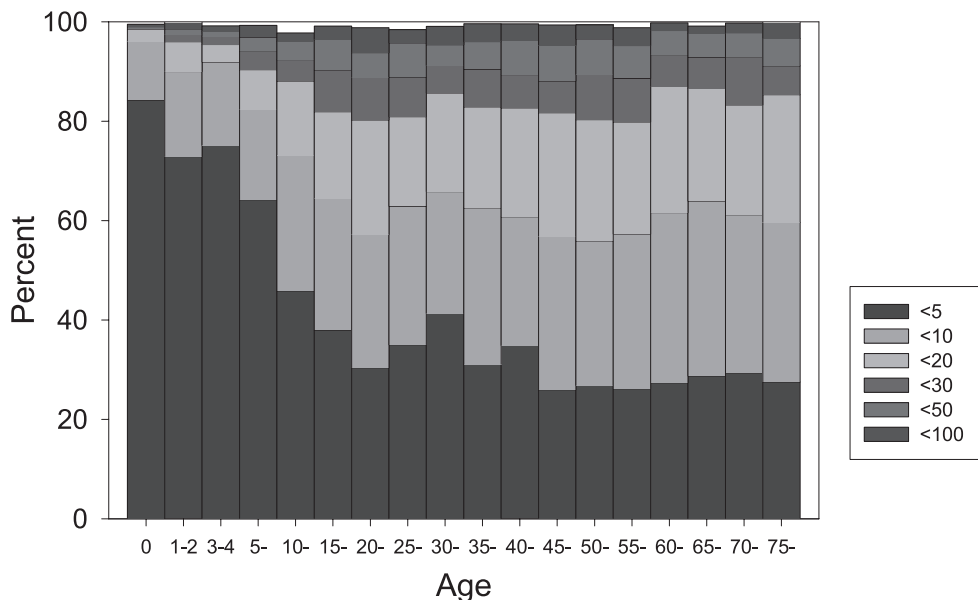
Fig 2: The age-specific IgG antibody levels in the population-based cross-sectional study in the Netherlands. Note that the categories are cumulative, i.e. individuals in the lower categories also belong to all higher categories.

A series of cut-off values was chosen (10, 20, 30, 40, 50, 60, 80, 100 and 150 U/ml) for which we calculated the (age-specific) incidence of infection with *B. pertussis* in the population. The estimated numbers of infected individuals were corrected for the discrepancy between the age-distribution of the cross-sectional study population and the age-distribution of the Dutch population in 1996 by weighting each one-year age class with an appropriate correction factor. The estimates of incidence of infection were limited to 3 to 79 year-olds. We did not estimate the incidence of infection in younger age-classes because IgG-PT in infants can be maternally derived and can be induced by vaccination (at the time of the study given in the Netherlands at 3,4,5 and 11 months of age). Above 3 years of age those factors do not influence the IgG-PT because maternally derived antibodies disappear within one year and IgG-PT induced by vaccination declines rapidly to very low or undetectable levels within one year after the last immunization [25, 26].

The average numbers of notified cases per 100,000 by age group in the period 1994 to 1996 were calculated using the age distribution of the Dutch population in 1994 to 1996. The period of 1994 to 1996 was chosen for reasons of comparability with the period for which the number of infections was estimated. For each age-class, the case to-infection ratio could then be estimated by dividing the average numbers (per 100,000) of notified cases by the age-specific estimates of incidence of infection.

To assess the influence of individual variation in responses on the uncertainty of the incidence estimates we chose to compute a confidence interval for every cut-off value based on the

following assumptions: (a) the longitudinal study population and the cross-sectional study population are the same in their responses to pertussis infection; (b) the variability in times since infection for different cut-off values is the same for the longitudinal and the cross sectional study population; and (c) the log times since infection are normally distributed with mean and standard deviation calculated from the log times since infection from the longitudinal study.

Results

We describe the results for some of the cut-off values, namely the values 40, 50, 80 and 100 U/ml, because those values are considered to be most conclusive for identifying recently infected individuals. After infection with *B. pertussis* the average time needed for the high IgG-PT level induced by infection to decline to 100 U/ml is 58.6 days (CI [54.2, 63.2]), to 80 U/ml it is 102.6 (CI [95.8, 109.8]) days, to 50 U/ml it is 208.9 (CI [195.4, 223.3]) days and to 40 U/ml it is 297.6 (CI [279.2, 317.2]) days (compare Figure 1). Using a cut-off level of 100 U/ml the estimated percentage of the population that has been infected in the past 58.6 days was 0.84% (i.e. 0.84% of the population sera contained IgG-PT of = 100 U/ml) resulting in an estimated incidence in the year before serum sampling of 5.2% (365.25 / 58.6 times 0.84%). A cut-off of 80 U/ml yielded an estimated incidence of 6.6% (365.25 / 102.6 times 1.86%), a cutoff value of 50 U/ml an estimated incidence of 6.6% and a cut-off value of 40 U/ml an estimated incidence of 7.2% in the year before serum sampling.

In summary, after standardization of the estimated incidences for various cut-off values to the time period of one year, one obtains remarkably consistent incidence estimates for a wide range of cut-off values (Figure 3).

The above results also hold for an age-stratified analysis, where the incidence of infection is estimated separately for each age category using the same range of cut-off levels of antibody titers. The consistency of the estimates for the yearly incidence of infection across different cut-off values indicates that the functional relationship between time since last infection and antibody titer as inferred from the longitudinal study in [23] is a useful description of the immune response to IgG-PT. In Figure 4 the estimated age-specific incidence of infection with *B. pertussis* in the population is shown as calculated for the cut-off of 50 U/ml. The estimated incidence of infection the year before blood sampling (1995-1996) amounted to 6571 per 100,000 (6.6%) on average for 3 to 79-years olds. This was 685 times higher than the incidence of notified cases in the period 1994 to 1996 of 9.6 per 100.000 (0.01%) for 3-79 year-olds (10.9 per 100.000 for all age groups), meaning that only 1 of 685 cases of infection is reported.

As shown in Figure 4, the estimated incidence of infection is considerably higher for all age groups in comparison to the incidence of notified cases (note the different scales).

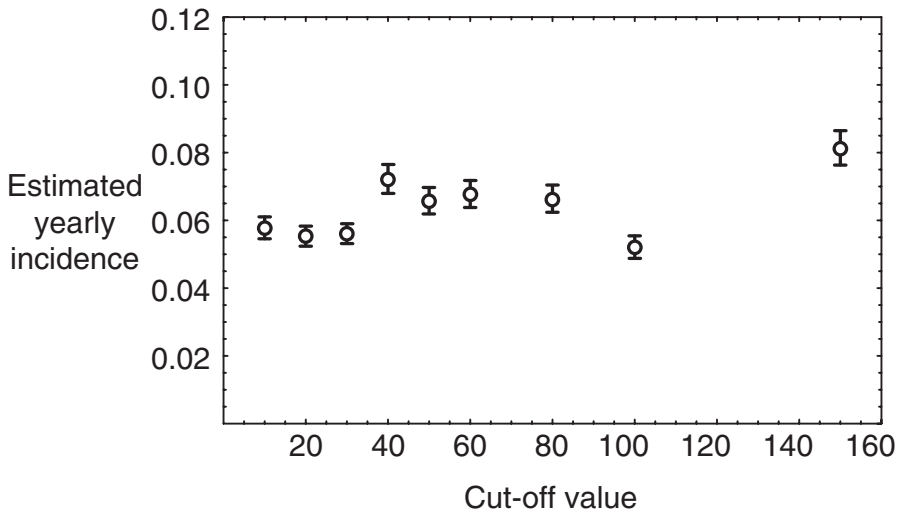*Fig 3:* The estimated yearly incidence (as a fraction of the population) for different cut-off values and confidence intervals.
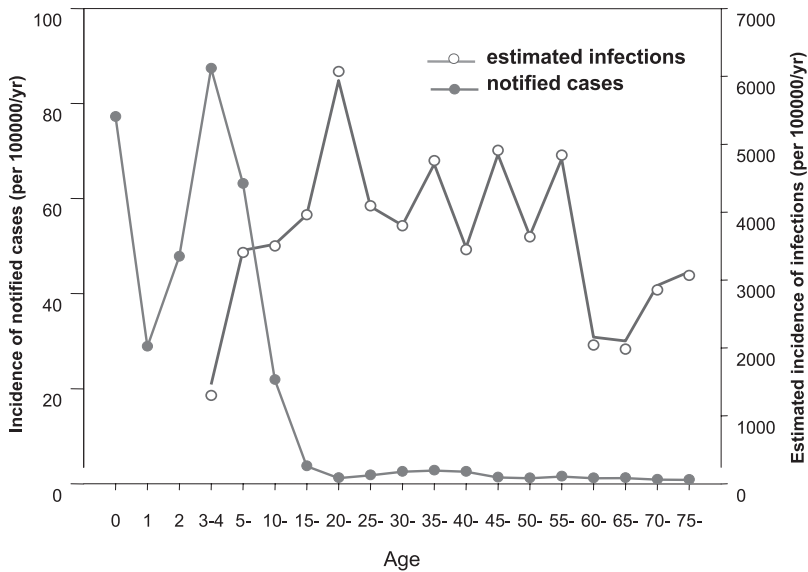

Fig 4: Estimated incidence of infection with *B. pertussis* per age group per 100.000 per year (open circles, right scale) and the annual incidence of notified cases of pertussis per age group per 100.000 averaged over the period 1994 to 1996 (filled circles, left scale).

The incidence of notified cases is high among 0-year-olds (77.2 per 100.000), 3-4 (87.4 per 100.000) and 5-9 year-olds (63.1 per 100.000) and decreases sharply till 20-24-years (1.2 per 100.000). For 30-44 year olds a somewhat higher reported incidence is observed (2.6-2.8 per 100.000). For those aged between 45 and 69 years the incidences fluctuate between 1.2 and 1.6 per 100.000, while the incidence is lowest for the oldest age groups (=70 years). In contrast, the estimated incidence of infection is lowest for 3-4-year olds (3299 per 100.000). It increases sharply up to the age of 20-24 years (10831 per 100.000) and decreases again afterwards to a level of around 6500 per 100000 in the age groups 25-59 years. In the oldest age groups (60 – 79 years) a further decrease to around 3500 per 100000 can be seen.

In Figure 5 the case-to-infection ratio by age group is shown. The ratio is highest for the youngest age-group - 1 out 38 infected children aged 3 to 4 is reported, - while it decreases afterwards. For 20 to 24-year olds the ratio is lowest, showing that only 1 out of 8966 infected cases are reported. For the remaining age groups it fluctuates between about 1 to 1500 and 1 to 6000. As the notification numbers for those age groups are small, there is a large uncertainty connected to those estimates.
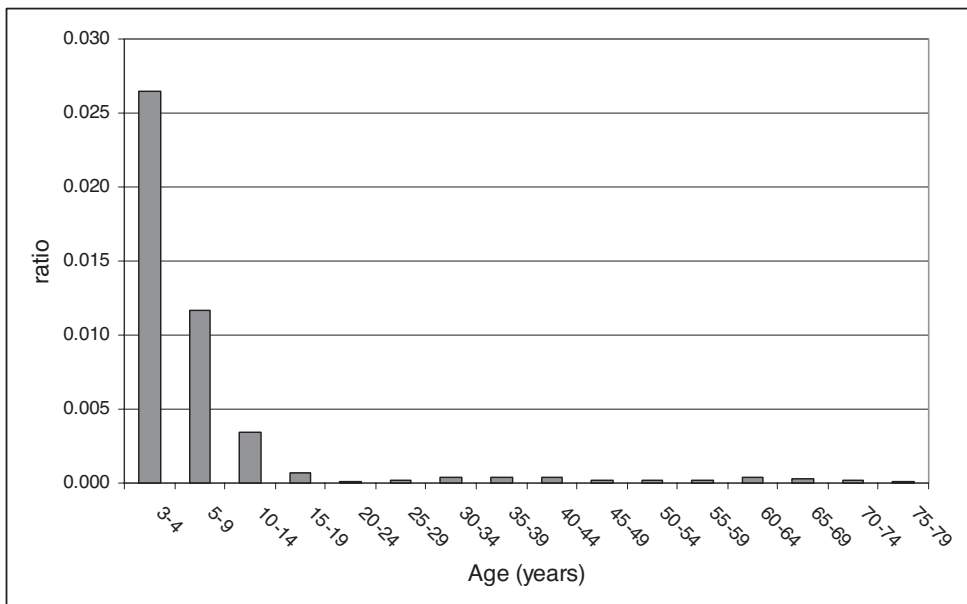

Fig 5: Ratio of incidence of notified cases to estimated infection incidence per age group.

Discussion

Incidence and age profile of infections

There has been a long-standing discussion in the literature, whether vaccination against infection with *B. pertussis* reduces transmission of infection as opposed to only reducing the incidence of clinical infections [27-29]. While there is agreement about the fact that vaccination does indeed reduce transmission, to estimate the amount of that reduction requires knowledge about the fraction by age of clinical cases among all infections. This fraction is determined by a complex interplay of the age-dependent force of infection, immunity and reporting behavior. Techniques of the analysis of serologic data have made it possible to identify individuals with recent *B. pertussis* infections and compare seroprevalence data with notifications of symptomatic disease [30-32]

IgG-PT levels in the Netherlands can be interpreted as markers of recent infection, because firstly the amount of pertussis toxin in the Dutch whole cell vaccine is very low and second, because vaccine-induced IgG-PT levels are minimal and short-lived [10]. However, also in populations in which pertussis vaccines are used that contain moderate to high amounts of pertussis toxin, vaccine induced IgG-PT declines to barely detectable levels within 2 to 4 years [33-36].

Two major issues clearly emerge from our analysis. Firstly, the estimated incidence of infections with *B. pertussis* is considerable in all age groups and much higher than the reported incidence [1]. We estimated that around 6.6% of the Dutch population had experienced infection with *B. pertussis* in the year before serum sampling, while, in contrast, the incidence of notifications in 1994 to 1996 amounted to 0.01% per year [1]. Secondly, the age-specific profile of the reported cases diverges remarkably from the estimated age-specific profile of incidence of infection with *B. pertussis*. While the highest incidence of reported symptomatic cases is observed among children aged 3 to 9 years, the incidence of infection is lowest among 3-4-year-olds, increases with age and peaks for 20 to 24-year-olds. Therefore, most cases are notified in those age-categories with the lowest incidence of infection.

Our cross-sectional study was performed in a limited time period (1995-1996). Therefore, the estimated incidence of infection and its age-distribution are a snapshot in time and it is not clear whether those estimates apply to other time periods. We believe that the age-specific profile of incidence of infection with *B. pertussis* is rather stable over time. Repeating the cross-sectional study can only assess whether or not this is correct. However, some support for our hypothesis is found in the similarity of the sero-profile of IgG-antibodies against pertussis toxin in 548 vaccinated children of 1-12 years in 1980, the sero-profile of about 800 individuals of all ages in 1992-1993 (pilot study for the large cross-sectional study) and the sero-profile in the present study in 1995-1996 [37]. Also, the fact that our findings in adolescents and adults

are in agreement with the high incidences found in prospectively followed cohorts of small numbers of adolescents and adults in the USA in other time period may be seen as supporting our hypothesis [6, 38, 39]. In those studies significant increases in sequential sera of IgA/IgG antibodies against pertussis toxin were taken to indicate infection with *B. pertussis*. In the studied cohorts incidences of 3.3% to 8% per year were estimated. As discussed by Teunis et al. [23], the immune response of IgG-antibodies against pertussis toxin after infection with *B. pertussis* shows large variation among individuals. Here, we worked with the point wise (for each titer value) averages of the individual response curves (Figure 1). This means that we neglected the dependence within individual responses but, as we applied this procedure to a large representative population sample, we assumed that variation on the individual level averages out on the population level. We assumed that the longitudinal study population and the cross-sectional study population are identical in their responses to pertussis infection. With the size of the longitudinal sample as it is available to us at present, we can only say that the responses in different patient categories did appear to be similar [23].

Several factors are responsible for the large discrepancies between reported pertussis cases and the estimated cases of infection in their incidences and age-profiles. The amount of underreporting varies by age, because severity of disease, medical care seeking and diagnostic power are varying with age. Indeed, a high rate of underreporting has been observed mainly in older children, adolescents and adults [7,9,12,13,40]. Recently, Strebel et al. performed active case finding among older children and adults (10-49 years) from a well-defined population, who consulted the physician with cough lasting at least 14 days [41]. An incidence of symptomatic infection with *B. pertussis* of 0.5% per year was found, which was about 100 fold higher than the incidence of notified cases in that age-category. A similar high incidence of symptomatic infections with *B. pertussis* among adults was found in a highly vaccinated region of France [42].

Another set of factors that influence the age-specific incidence profile of pertussis infections is related to the dynamics of transmission and immunity. The transmission of air-borne infections is strongly determined by the age-dependent patterns of mixing in a population [43]. The contact rates between age groups, in conjunction with age-specific fractions of susceptible, infectious and immune individuals, channel the transmission of the infection through the population. The peaks observed in Figure 4 of age-specific incidence in the population might be related to high contact rates, the lower infection rate for those aged 60 years and older to a lower contact rate in that age group. The gradual increase with age of the incidence of infection to a peak of 10.8% in 20-24 year-olds suggests that there is a high variability in the duration of vaccine-induced immunity, which in some may be less than 2 years, in others more than 5 years. However, the incidence of notified cases of pertussis is highest among 3-9 year-olds, suggesting a strong reporting bias in that age category but also showing that susceptibility for symptomatic infection with *B. pertussis* may re-emerges

shortly after vaccination. In the USA, sero-prevalence data of IgG-PT in the population have been seen as an indication that immunity after vaccination with whole-cell vaccines wanes after about 10 years [44]. However, in that study the investigators did not take the decline of IgG-PT after infection into account.

The mean incidence of 6.6%, if constant in time, indicates that on average, within a period of 20 years the entire population experiences infection, i.e. that vaccination against pertussis will be followed on average by 3 episodes of natural infection during life. Indeed, there are strong indications that immunity wanes also after natural infection and that re-infection is possible. German investigators estimated the duration of the protective period following natural infection at 20 years [45]. Recently, we observed laboratory-confirmed mildly symptomatic infection with *B. pertussis* in 4 children 3,5-12 years after the first laboratory-confirmed clinically typical episode of pertussis [46].

Implications for vaccination

In the Netherlands, the age-specific profile of notifications with the highest incidence in those aged 3 to 9 years led to the decision of the Dutch Health Council to introduce a booster vaccination with acellular pertussis vaccine at 4 years of age [47]. Since siblings play a role in transmission to vulnerable infants [14, 15], one expects the incidence of severe pertussis in infants to decline. However, on the basis of the incidence estimates presented here, we expect that the introduction of the booster vaccination at 4 years on the long run will lead to a shift of the peak of infections to older age groups. Those older age groups, being the peak child bearing ages, may have more contacts with vulnerable infants, implying that on the long run booster vaccination might lead to an increase of the incidence of severe pertussis in infants. This is consistent with the fact that in the USA, where a booster vaccination at preschool age has been included in the vaccination-scheme for several decades, the proportion of infants among the notifications of pertussis is relatively high [4].

Conclusion

More insight is needed into the role of adults as compared to siblings in the transmission of *B. pertussis* to young unvaccinated infants. The results of the present study support the findings of others in that adults are an important source of infection [8,12-14,16]. More effective than booster vaccinations for adults given in 10 yearly intervals might be a strategy that directly targets (future) parents and caregivers [11]. Mathematical modeling studies are needed for a precise quantitative analysis of the effects of different vaccination strategies on the age-specific incidence of (symptomatic and asymptomatic) pertussis infections. Previous modeling studies [48, 49] had to cope with the lack of data concerning the force of infection and the age dependent fractions of symptomatic and notified cases of infection. While we are still far from having a solid quantitative basis on which to build reliable mathematical models,

we think that our study investigated an essential link between the transmission dynamics as described by mathematical models and notification data of pertussis infections. To our knowledge, the methodology we used to estimate the frequency of infection with *B. pertussis* has not previously been described and can be used more generally to estimate infection frequency from sero-prevalence data.

References

1. Melker HE de, Schellekens JFP, Neppelenbroek SE, Mooi FR, Rümke HC, Conyn van Spaendonck MAE. Reemergence of pertussis in the highly vaccinated population of the Netherlands: observations on surveillance data. Emerg Infect Dis 2000; 6: 348-57.

2. De Serres G, Boulianne N, Douville Fradet M, Duval B. Pertussis in Quebec: ongoing epidemic since the late 1980s. Can Commun Dis Rep 1995; 21: 45-8.

3. Andrews R, Herceg A, Roberts C. Pertussis notifications in Australia, 1991 to 1997. Commun Dis Intell 1997; 21: 145-8.

4. Güris D, Strebel PM, Bardenheier B, Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 19901996. Clin Infect Dis 1999; 28: 1230-7.

5. Senzilet LD, Halperin SA, Spika JS, Alagaratnam M, Morris A, Smith B and the Sentinel Health Unit Surveillance System Pertussis Working Group. Pertussis is a frequent cause of prolonged cough illness in adults and adolescents. Clin Infect Dis 2001; 32: 1691-7.

6. Cromer BA, Goydos J, Hackell J, Mezzatesta J, Dekker C, Mortimer EA. Unrecognized pertussis infection in adolescents. Am J Dis Child 1993; 147: 575-7.

7. Yih WK, Lett SM, des Vignes FN, Garrison KM, Sipe PL, Marchant CD. The increasing incidence of pertussis in Massachusetts adolescents and adults, 1989-1998. J Infect Dis 2000; 182: 1409-16.

8. Cherry JD. The role of *Bordetella pertussis* infections in adults in the epidemiology of pertussis. Develop Biol Stand 1997; 89: 181-6.

9. De Serres G, Shadmani R, Duval B, et al. Morbidity of pertussis in adolescents and adults. J Infect Dis 2000; 182: 174-9.

10. Melker HE de, Versteegh FGA, Conyn-van Spaendonck MAE, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. J Clin Microbiol 2000; 38: 800-6.

11. Gardner P. Indications for acellular pertussis vaccines in adults: the case for selective rather than universal recommendations. Clin Infect Dis 1999; 28; S131-5.

12. Nelson JD. The changing epidemiology of pertussis in young infants: the role of adults as a reservoir of infection. Am J Dis Child 1978; 132: 371-3.

13. Deen JL, Mink CAM, Cherry JD et al. Household contact study of *Bordetella pertussis* infections. Clin Infect Dis 1995; 21: 1211-9.

14. Baron S, Njamkepo E, Grimprel E et al. Epidemiology of pertussis in French hospitals in 1993 and 1994: thirty years after a routine use of vaccination. Pediatr Infect Dis J 1998; 17: 412-8.

15. Srugo I, Benilevi D, Madeb R, et al. Pertussis infection in fully vaccinated children in day-care centers, Israel. Emerg Infect Dis 2000; 6: 526-9.

16. Grimprel E, Baron S, Lévy-Bruhl D, et al. Influence of vaccination coverage on pertussis transmission

in France. Lancet 1999; 354: 1699-1700.

17. Long SS, Welkon CJ, Clark JL. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis 1990; 161; 480-6.

18. Elliot E, McIntyre P, Ridley G, et al. National study of infants hospitalized with pertussis in the acellular vaccine era. Pediatr Infect Dis 2004; 23: 246-52.

19. Izurieta HS, Kenyon TA, Strebel PM, Baughman AL, Shulman ST, Wharton M. Risk factors for pertussis in young infants during an outbreak in Chicago in 1993. Clin Infect Dis 1996; 22: 503-7.

20. Edwards K. Pertussis: an important target for maternal immunization. Vaccine 2003; 21: 3483-6.

21. Müller FM, Hoppe JE, Wirsing-von König CH. Laboratory diagnosis of pertussis: state of the art in 1997. J Clin Microbiol 1997; 35: 2435-43.

22. Halperin SA, Bortolussi R, Kasina A, Wort AJ. Use of a Chinese hamster ovary cell cytotoxicity assay for the rapid diagnosis of pertussis. J Clin Microbiol 1990; 28: 32-8.

23. Teunis PFM, Heijden OG van der, Melker HE de, Schellekens JFP, Versteegh FGA, Kretzschmar M. Kinetics of IgG-antibody response to pertussis toxin after infection with B. pertussis. Epidemiol Infect. 2002; 129 (3): 479-89.

24. Melker HE de, Conyn-van Spaendonck MAE. Immunosurveillance and the evaluation of national immunisation programmes: a population-based approach. Epidemiol Infect 1998; 121: 637-43.

25. Nagel J, Graaf de S, Schijf-Evers D. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LFP antibody levels. Develop Biol Standard 1985; 61: 325-30.

26. Labadie J, Sundermann LC, Rümke HC and the DTP-IPV~Hib vaccine study group. Multi-center study on the simultaneous administration of DTP-IPV and Hib PRP-T vaccines. Part 1. Immunogenicity. National Institute of Public Health and the Environment. Report No. 124001003, Bilthoven, The Netherlands, 1996.

27. Fine PEM, Clarkson JA. The recurrence of whooping cough: possible implications for assessments of vaccine efficacy. Lancet 1982; 1: 666-9.

28. Rohani P, Earn DJD, Grenfell BT. Impact of immunisation on pertussis transmission in England and Wales. Lancet 2000; 355: 285-6.

29. Gay NJ, Miller E. Pertussis transmission in England and Wales. Lancet 2000; 355: 1553-4.

30. Mink CM, Cherry JD, Christenson PD et al. A search for *Bordetella pertussis* infection in university students. Clin Infect Dis 1992; 14: 464-71.

31. Nardone A, Pebody RG, Maple PAC, Andrews N, Gay NJ, Miller E. Seroepidemiology of *Bordetella pertussis* in England and Wales. Vaccine 2004; 22: 1314-9.

32. Pebody RG, Gay NJ, Giammanco A et al. The seroepidemiology of *Bordetella pertussis* infection in Western Europe. Epidemiol Infect 2005; 133: 159-71

33. Cherry J, Beer T, Chartrand A, et al. Comparison of values of antibody to *Bordetella pertussis* antigens in young German and American men. Clin Infect Dis 1995; 20: 1271-4.

34. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to

protection after household exposure to *Bordetella pertussis*. Vaccine 1998; 16: 1907-16.

35. Halperin SA, Scheifele D, Barreta L, et al. Comparison of a fifth dose of a fivecomponent acellular or a whole cell pertussis vaccine in children four to six years of age. Pediatr Infect Dis J 1999; 18: 772-9.

36. Pichichero ME, Edwards KM, Anderson EL, et al. Safety and immunogenicity of six acellular pertussis vaccines and one whole-cell pertussis vaccine given as a fifth dose in four- to six-year-old children. Pediatrics 2000; 105: e11.

37. Melker HE de, Boshuis HGL, Mommers MPTM, et al. Serodiagnostiek van kinkhoest: interpretatie van hoge IgG/IgA concentraties in acute fase serum: (Her)evaluatie van eenpuntsserologie. RIVM-report No. 128507003. Bilthoven, 1996.

38. Deville JG, Cherry JD, Christenson PD, et al. Frequency of unrecognized *Bordetella pertussis* infections in adults. Clin Infect Dis 1995; 21: 639-42.

39. Hodder SL, Cherry JD, Mortimer EA Jr., Ford AB, Gornbein J, Papp K. Antibody responses to *Bordetella pertussis* antigens and clinical correlations in elderly community residents. Clin Infect Dis 2000; 31: 7-14.

40. Orenstein WA. Pertussis in adults: epidemiology, signs, symptoms and implications for vaccination. Clin Infect Dis 1999; 28: S147-50.

41. Strebel P, Nordin J, Edwards K, et al. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995-1996. J Infect Dis 2001; 183: 1353-9.

42. Gilberg S, Njamkepo E, Parent du Chatelet I, et al. Evidence of *Bordetella pertussis* infection in adults presenting with persistent cough in a French area with very high whole-cell vaccine coverage. J Infect Dis 2002; 186: 415-8.

43. Wallinga J, Edmunds WJ, Kretzschmar M. Human contact patterns and the spread of airborne infectious diseases. Trends in Microbiology 1999; 7: 372-7.

44. Cattaneo LA, Reed GW, Haase DH, Wills MJ, Edwards KM. The seroepidemiology of *Bordetella pertussis* infections: a study of persons aged 1-65 years. J Infect Dis 1996; 173: 1256-9.

45. Wirsing von Konig CH, Postels-Multani S, Bock HL, Schmitt HJ. Pertussis in adults: frequency of transmission after household exposure. Lancet 1995; 346: 1326-9.

46. Versteegh FGA., Schellekens JFP, Nagelkerke AF, Roord JJ.. Laboratory confirmed re-infection with *Bordetella pertussis*. Acta Paediatrica 2002; 91: 95-7.

47. Dijk van GD. Invoering extra kinkhoest-vaccinatie. Infectieziekten Bulletin 2000; 11: 177-8.

48. Boven van M, Melker HE de, Schellekens JPF, Kretzschmar M. A model based evaluation of the 1996-7 pertussis epidemic in the Netherlands. Epidemiol. Infect. 2001; 127: 73-85.

49. Rie A van, Hethcote HW. Adolescent and adult pertussis vaccination: computer simulations of five new strategies. Vaccine 2004; 22: 3154-65.

Chapter 7

Kinkhoest en Zwangerschap

F.G.A. Versteegh [1], B. Muller [1], J.C.M. van Huisseling [2], J.F.P. Schellekens [3], J.J. Roord [4]

1: Groene Hart Ziekenhuis, afdeling Kindergeneeskunde, Gouda

2: Groene Hart Ziekenhuis, afdeling Gynaecologie/Verloskunde, Gouda

3: Rijksinstituut Voor Volksgezondheid en Milieu, Bilthoven

4: Vrije Universiteit Medisch Centrum, afdeling Kindergeneeskunde, Amsterdam

Abstract

Whooping cough is a serious, contagious disease. With the recent increase in *Bordetella pertussis* infections in the vaccinated population the risk for perinatal whooping cough (in mother and child) has increased. The infants too young to be vaccinated are at greatest risk for severe complications and fatal outcome of pertussis. Because *Bordetella pertussis* infections in adults ànd in young infants may present as atypical disease the diagnosis may be missed in first instance. Symptoms, diagnosis, treatment and prophylaxis are discussed. The purpose of this paper is to improve knowledge about the risks and prevention of perinatal pertussis in mother and child.

Inleiding

Kinkhoest, een zeer besmettelijke luchtweginfectie met de obligaat humane bacterie *Bordetella pertussis (Bp)*, is, ondanks vaccinatie, blijven voorkomen en de incidentie lijkt toe te nemen, vooral bij gevaccineerde kinderen en volwassenen. [1,2] Ook het aantal gevallen onder de leeftijd van 3 maanden neemt toe, met een mortaliteit van 1-2 %. [2,3] Kinkhoest bij pasgeborenen vindt bovendien vaker plaats dan wordt herkend [4]. Het probleem van perinatale kinkhoest wordt onderschat, alhoewel er de laatste jaren toenemend aandacht voor is. [4-16] Tegen deze achtergrond is het voor de gynecoloog/obstetricus belangrijk om te beseffen dat zwangeren die kinkhoest in de laatste maanden van hun zwangerschap doormaken hun pasgeboren kind kunnen besmetten en dat bij een bewezen infectie behandeling van de zwangere en preventieve behandeling van de pasgeborene geïndiceerd is. Het doel van dit artikel is obstetrici en verloskundigen van een belangrijk en mogelijk toenemend probleem bewust te maken door op de grote risico's van perinatale *Bordetella pertussis* infectie te wijzen en gegevens wat betreft symptomen, diagnose, behandeling en profylaxe op een rijtje te zetten.

Etiologie, Vaccinatie, Incidentie:
Kinkhoest bij de mens wordt meestal veroorzaakt door Bp, soms door de *Bordetella parapertussis*. De bacterie hecht zich aan het trilhaarepitheel van de nasopharynx, waarna hij zich lokaal vermenigvuldigt en toxinen produceert die schade in de luchtwegen aanrichten waardoor de typische uitputtende, aanvalsgewijze hoest ontstaat. De bacterie zelf is niet invasief. Wel kunnen er systemische effecten van geabsorbeerde toxines optreden, zoals een sterke leucolymfocytose.
Sinds 1954 worden in Nederland kinderen op de leeftijd van 3, 4, 5 en 11 maanden gevaccineerd tegen kinkhoest met een whole cell vaccin dat geproduceerd wordt door het RIVM. Sinds 1999 zijn de eerste drie immunisaties vervroegd van 3, 4 en 5 naar 2, 3 en 4 maanden, om de

kwetsbaarheid van de jongste kinderen zo klein mogelijk te maken. Sinds 2000 is bovendien een boostervaccinatie met een acellulair vaccin op 4 jarige leeftijd ingevoerd, om de circulatie van Bp en ziektegevallenbij lagere schoolkinderen te verminderen. Wel heeft de Inspectie voor de Gezondheidszorg zeer recent zijn grote zorg uitgesproken over de toenemende kritische houding van ouders tegen het vaccineren o.a. onder invloed van de Nederlandse Vereniging Kritisch Prikken.

Noch vaccinatie, noch een infectie levert een blijvende immuniteit op. Infecties met Bp, hetzij symptoomloos, hetzij met milde atypische symptomen, hetzij met typische symptomen (kinkhoest) kunnen dus ook op latere leeftijd optreden. Ook mensen die een infectie met Bp hebben doorgemaakt kunnen later opnieuw die infectie krijgen, vaak gemitigeerd en deels symptoomloos. [17-23] Waarschijnlijk maakt ruim 6 % van de bevolking jaarlijks een infectie met Bp door. [24]

Nederland heeft al decennia lang een hoge vaccinatiegraad (ongeveer 96%). Desondanks worden jaarlijks 300 tot 500 kinderen in het ziekenhuis opgenomen wegens kinkhoest(60% jonger dan 3 maanden, 18% tussen 3 en 12 maanden). Vrijwel alle sterfgevallen ten gevolge van kinkhoest (in Nederland 2-5 per jaar volgens registratie van het CBS) vallen in de groep jonger dan 3 maanden.[2,3]

Symptomatologie:

Het typische ziektebeeld kenmerkt zich door drie stadia. Het eerste stadium is het catarrale stadium dat een à twee weken duurt en wordt gekarakteriseerd door niet specifieke symptomen van milde hoest, verkoudheid en soms lichte koorts. Dit is de meest besmettelijke fase. Hierna volgt het paroxysmale stadium dat zich kenmerkt door plotseling heftige en herhaaldelijke hoestaanvallen met de typische gierende inademing aan het einde van de paroxysme. Deze periode kan enkele weken tot maanden duren. Vervolgens treedt het convalescentie stadium in met een geleidelijke afname van de klachten.

Kinkhoest en Zwangerschap:

De belangrijkste bron voor een infectie met Bp zijn vermoedelijk volwassenen met geringe symptomen die kinderen in hun omgeving besmetten. Waarschijnlijk zijn de moeders de belangrijkste infectiebron voor kinderen, en met name pasgeborenen. Maar ook andere gezinsleden kunnen als infectiebron fungeren.

Het doormaken van een infectie met Bp geeft geen levenslange bescherming. [17] De antistoffen verdwijnen na een infectie met Bp relatief snel. Na een jaar is de gemiddelde waarde voor IgG-PT 32 U/ml. [25] Onbekend is op welk niveau er geen bescherming meer is, echter algemeen wordt aangenomen dat bij een titer lager dan 20 U/ml er onvoldoende bescherming is. Indien de moeder antistoffen opbouwt tegen Bp, tijdens de zwangerschap of kort daarvoor, gaan deze via de placenta over naar het kind. [26-31] Uit de beschikbare data blijkt, dat indien

de infectie met Bp vroeg genoeg in de zwangerschap plaatsvindt en de moeder voldoende tijd heeft om antistoffen te vormen, er weinig risico is voor het kind. [9,10] Indien de infectie met Bp bij moeder optreedt kort voor de partus (laatste 1 à 2 maanden), is er onvoldoende bescherming voor de baby en als de besmetting bij moeder optreedt na de bevalling, is er evenmin bescherming en ook niet als de infectiebron een van de anderen gezinsleden is (of een van de bezoekers). [4,6,7,16,32]

Dat maternale antistoffen bescherming aan het kind kunnen bieden is in verschillende studies aangetoond. Al in 1943 toonden Cohen en Scadron [31] aan dat antistoffen van moeders die tijdens de zwangerschap werden gevaccineerd tegen kinkhoest overgingen naar de pasgeborenen. Oda laat in muizen zien dat babymuizen geboren uit gevaccineerde moeders beschermd waren tegen Bp infectie en dat ook colostrum een dergelijk beschermend effect heeft. [27,30] Ook Arciniega laat zien dat antistoffen tegen Bp in muizen passieve bescherming kunnen geven en dat deze antistoffen ook gevonden worden in de sera van pasgeborenen. [26] Er zijn slechts enkele studies beschreven betreffende kinkhoest gedurende de zwangerschap. [9,10,23,32] Meestal verloopt de ziekte voor de moeder zonder al te veel problemen. [9] Gedurende een langetermijn studie van zwangere vrouwen met een besmettelijke ziekte tijdens de bevalling vonden Granström et al in de periode 1975-1985 35 vrouwen met kinkhoest, waarvan 19 in 1984-1985. Kinkhoest bleek daarmee in de laatste twee jaar van die studie de meest voorkomende "kinderziekte" bij zwangere vrouwen te zijn. [23] MacLean en Calder [9] beschrijven 4 gevallen van kinkhoest in de zwangerschap van de 43 zwangerschappen (9,3%) die zich in die periode voordoen in een praktijk in Schotland.

Als besmetting van de pasgeborene tijdens of kort na de bevalling heeft plaatsgevonden ontwikkelt het ziektebeeld zich meestal rond de tweede, derde week. [4,6,7,11,13,14,16,32] Besmetting in utero is wel eens vermoed, maar nooit bewezen. [6] De klachten bestaan uit voedingsproblemen, snelle ademhaling en hoesten. Bij de jongste kinderen kan echter het klassieke ziektebeeld van kinkhoest achterwege blijven en staan algeheel ziek zijn en apneus op de voorgrond. Ook bradycardiën kunnen het eerste symptoom zijn. [6,13] Deze aspecifieke presentatie is waarschijnlijk ook de reden dat het ziektebeeld vaak niet of laat herkend wordt bij hele jonge kinderen.

Het ziektebeeld wordt mogelijk verergerd als er sprake is van dubbelinfecties. [33] Beiter [32] beschrijft een casus, waarbij de kinkhoest bij moeder niet werd herkend, de baby een week na de geboorte ziek wordt en overlijdt. Er bleek sprake van een dubbelinfectie met adenovirus. MacLean [9] vindt bij 1 van zijn 4 beschreven patiënten één co-infect met Influenza B, en Smith et al [11] beschrijven 9 kinderen met ernstige vroege kinkhoest, waarvan 4 met een virale co-infectie. Van de 9 baby's overlijden er 6, waarvan 3 met een co-infectie.

De incidentie van perinatale pertussis in Nederland is onbekend echter uitgaande van tweehonderd duizend partussen per jaar in Nederland en een infectiefrequentie bij volwassenen van ongeveer 6% per jaar (zie hoofdstuk 6), zou dit betekenen dat er per jaar

ongeveer negenduizend vrouwen een infectie met Bp doormaken in de zwangerschap. Dit wil zeggen dat ongeveer 3000 vrouwen een infectie met Bp doormaken in de laatste twee maanden van de zwangerschap en de eerste maand erna. Zoals beschreven hoeft het klinisch beeld niet altijd even duidelijk te zijn en kan variëren van een milde luchtweginfectie tot typische kinkhoestklachten. Daartegenover staat dat ook andere verwekkers van luchtweginfecties een kinkhoestachtig beeld kunnen veroorzaken. [34,35]

Gezien de hoge morbiditeit en mortaliteit van perinatale pertussis bij de neonaat is het belangrijk, zowel in de laatste weken van de zwangerschap als rondom de partus, bedacht te zijn op het ziektebeeld kinkhoest bij de moeder en na afname van diagnostiek moeder en kind direct te gaan behandelen.

Diagnostiek:

De diagnostiek bij volwassenen en oudere kinderen met een verdenking op een infectie met Bp bestaat uit nasopharyngeale kweek en/of PCR op een nasopharyngeale wat en/of serologie.[36] De kweek is erg gevoelig maar slechts korte tijd betrouwbaar af te nemen, 1 à 2 weken na de eerste ziektedag. Voor de PCR geldt hetzelfde, al is de afnameperiode 3 tot 4 weken na de eerste ziektedag. Serologie wordt pas enige weken na de eerste ziektedag positief en is daarom in de acute fase niet behulpzaam. Bij baby's dient ongeacht de ziekteduur altijd naast de serologie een kweek en/of PCR te worden ingezet, aangezien bij hen de afweer tegen Bp langzamer op gang komt en er langer een kans bestaat op een positief resultaat. Daarnaast zijn er ook andere ziekteverwekkers (o.a. adenovirus, influenzavirus, para influenzavirus, respiratoir syncytieel virus, *Mycoplasma pneumoniae*), die vergelijkbare hoestbeelden kunnen geven. Bovendien geven co-infecties waarschijnlijk meer morbiditeit. Voordat de uitslagen van de diagnostiek echter bekend zijn gaat er enige tijd voorbij. Het is dan ook belangrijk na afname van diagnostiek snel met behandeling te beginnen. [10]

Behandeling: [10,16,28,29,37-40]

Medicamenteus:

Granström et al [10] adviseren de moeder te behandelen met Erythromycine 3 keer daags 250 tot 500 mg gedurende 10 dagen. Dit is niet nodig als de klachten langer dan 7 weken geduurd hebben op moment van diagnose, omdat het dan zelden meer voorkomt dat de patiënt nog Bp bij zich draagt en besmettelijk is. Wanneer de bevalling plaatsvindt binnen 7 weken na het ontstaan van de kinkhoest wordt geadviseerd bij opname een Erythromycine kuur te geven, zelfs als de moeder al een Erythromycine kuur gehad heeft. Dit omdat er geen 100 % zekerheid is dat de eerste kuur de Bp volledig heeft verwijderd. Ook de nieuwe macroliden (clarithromycine, azithromycine) zijn effectief gebleken in de behandeling van kinkhoest. [40]

De bevalling moet gebeuren in een eenpersoons verloskamer en na de bevalling dienen moeder en kind op een eenpersoonskamer behandeld te worden. Het kind van de moeder

met kinkhoest krijgt, gedurende 5 dagen na de geboorte, 40 tot 50 mg. Erythromycine per kilo lichaamsgewicht. [10,16] Er is geen bezwaar tegen borstvoeding. Voordat moeder en kind naar huis ontslagen worden dienen alle gezinsleden, die mogelijk verdacht kunnen worden van een kinkhoestinfect, ook behandeld te worden met Erythromycine, gedurende minstens 5 dagen, omdat zij als mogelijke infectiebron een risico kunnen vormen voor de pasgeborene. Een probleem hierbij is natuurlijk dat bij volwassenen de kinkhoestinfectie praktisch symptoomloos of met atypische symptomen kan verlopen, zodat het moeilijk kan zijn mensen, die een risico vormen in de omgeving van het kind, te identificeren.

Behandeling met pertussis immunoglobuline lijkt succesvol, maar wordt niet op grote schaal gebruikt en is in Nederland niet beschikbaar. [37,38]

Preventie:

Kinkhoest is, ondanks de bestaande vaccinatie programma's, nog steeds een probleem, met name voor de heel jonge kinderen die niet of onvolledig gevaccineerd zijn. Om te komen tot een wereldwijde aanpak is de Global Pertussis Initiative opgericht, een internationale samenwerking van deskundigen vanuit verschillende disciplines . [41]

Er zijn verschillende vaccinatie strategieën na het eerste levensjaar mogelijk voor de preventie van kinkhoest [41]:

-het vaccineren van alle volwassenen: hiervoor is het in veel landen (ook Nederland) nog nodig een infrastructuur op te zetten om alle volwassenen hiervoor te motiveren en vervolgens te kunnen vaccineren. Voor een effectieve reductie van het aantal kinkhoest gevallen bij jonge kinderen is waarschijnlijk een vaccinatiegraad van >85% nodig en moet de vaccinatie iedere 10 jaar herhaald worden. [42,43] Dit wordt al aanbevolen in Canada en Oostenrijk.

-het vaccineren van alle adolescenten: omdat de immuniteit door vaccinatie tegen kinkhoest sterk gedaald is na 10-12 jaar vormen ook adolescenten een belangrijke bron voor pertussis infectie. De geschatte incidentie van kinkhoest in de leeftijdsgroep van 20-24 jaar is het hoogst van alle leeftijden (bijna 11%)(zie hoofdstuk 6). Revaccinatie alleen bij adolescenten (en niet bij volwassenen) zal onvoldoende bescherming bieden. Ook hier geldt dat in veel landen eerst een programma opgezet zal moeten worden om ook adolescenten te motiveren en vervolgens te vaccineren. Dergelijke programma's worden aanbevolen in b.v. Australië, België, Canada, Duitsland, Finland, Frankrijk, Oostenrijk en Zwitserland.

-revaccineren op de leeftijd van 4-6 jaar: het revaccineren van kinderen in deze leeftijdsgroep gebeurt al in veel landen, in Nederland sinds 2000. Met het opnieuw vaccineren van kinderen rond het 4^e jaar wordt verwacht dat de immuniteit tegen kinkhoest zal aanhouden tot verder in de adolescentie.

-selectief vaccineren van aanstaande moeders, familie en nauwe contacten: vaccinatie van zwangeren in het 3^e trimester met de nieuwe acellulaire vaccins staat op dit moment ter discussie, mede gezien de onbekende risico's voor de foetus. [29] Middels een dergelijke vaccinatie zouden

echter wel beschermende antistoffen aan het kind kunnen worden overgedragen, zoals bij een natuurlijke infectie, die mogelijk voldoende bescherming zouden kunnen bieden gedurende de eerste twee maanden tot de eerste vaccinatie. Een andere mogelijkheid is moeder direct post partum te vaccineren, en familieleden en andere nauwe contacten ook, deze zo mogelijk al voor de bevalling. Ook kan nog overwogen worden vrouwen met kinderwens te vaccineren voor de zwangerschap.

-het vaccineren van personeel werkzaam in de gezondheidszorg of kinderopvang: Ziekenhuispersoneel loopt ook het risico kinkhoest op te lopen, zowel thuis als in het ziekenhuis, door patiënten met kinkhoest. Granström et al [10] geven aan dat voor medisch personeel het risico kinkhoest te krijgen van opgenomen patiënten met kinkhoest minimaal is. Ze adviseren wel dat medewerkers met hoestklachten niet zouden moeten mogen zorgen voor patiënten (moeders en pasgeborenen) totdat een diagnose is gesteld. [10] Ook in de recente studie van Riffelmann et al blijkt dat er geen hogere antistoftiters voorkomen bij ziekenhuis personeel in vergelijking met bloeddonors en matrozen, echter zij geven niet aan of er ten tijde van het onderzoek patiënten met Bp infecties waren opgenomen. [44] Kurt et al [12] en Linnemann et al [45] laten echter zien dat personeel wel degelijk kinkhoest kan overbrengen op patiënten. Weber et al [40] geven een overzicht van verschillende studies waarin kinkhoest epidemieën in ziekenhuizen worden beschreven, veroorzaakt door patiënten, ziekenhuis medewerkers en bezoekers. Er is dus oplettendheid geboden ten aanzien van hoestklachten in het algemeen en kinkhoest in het bijzonder bij ziekenhuispersoneel.

-verbeteren/aanpassen huidige vaccinatieschema bij jonge kinderen: Vaccinatie van de neonaat kort na de geboorte heeft weinig zin. Englund et al laten zien dat een preëxistent hoger gehalte aan maternale antistoffen tegen kinkhoest bij het jonge kind een verminderde antistofreactie geeft na vaccinatie met een whole cell vaccin, maar niet bij een acellulair vaccin [28]. Bovendien zijn pasgeborenen nog niet goed in staat antistoffen te vormen.

Conclusies en Aanbevelingen:
1. Het niet overwegen van een infectie met Bp bij hoogzwangere vrouwen met luchtweginfecties kan fatale gevolgen hebben voor de pasgeborene.
2. Een infectie met Bp kan voorkomen bij volwassenen ook al zijn zij gevaccineerd of hebben zij eerder een infectie met Bp doorgemaakt. Bovendien kan een infectie met Bp bij volwassenen gemitigeerd en zelfs onherkenbaar verlopen.
3. Als de moeder geen infectie met Bp gedurende de zwangerschap of het jaar ervoor heeft doorgemaakt zijn er waarschijnlijk geen of onvoldoende beschermende antistoffen die transplacentair kunnen worden overgedragen.
4. Het klinisch beeld van een infectie met Bp bij pasgeborenen verschilt van het klassieke kinkhoestbeeld en wordt daardoor vaak laat of niet herkend.
5. Bij (verdenking op) perinatale pertussis bij de moeder dienen moeder èn kind

adequaat behandeld te worden evenals naaste familie. Overwogen dient te worden ook (para)medisch personeel dat in nauw contact is geweest met de patiënt te behandelen.

6. Andere infectieuze verwekkers kunnen een kinkhoestachtig beeld veroorzaken.

7. Dubbelinfecties van een infectie met Bp met een andere verwekker kunnen een ernstiger beeld laten zien.

8. Om de bescherming van pasgeborenen tegen kinkhoest te verbeteren zijn nieuwe vaccinatiestrategieën nodig.

Referenties

1. Melker HE de, Schellekens JFP, Neppelenbroek SE, Conyn-van Spaendonck MAE: Epidemiologie van kinkhoest in Nederland: observaties op grond van surveillancegegevens. Ned Tijdschr Med Microbiol 2000;8:101-108

2. Greeff SC de, Melker HE de, Schellekens JFP, Conyn-van Spaendonck MAE: Pertussis; description and evaluation based on surveillance data of 1999 and 2000. RIVM report 128507009/2001

3. Nelson JD: The changing epidemiology of pertussis in young infants. The role of adults as reservoirs of infection. Am J Dis Child 1978;132:371-373

4. McGregor J, Ogle JW, Curry-Kane G: Perinatal pertussis. Obstet Gynecol 1986;68:582-586

5. Congeni BL, Orenstein DM, Nankervis GA: Three infants with neonatal pertussis. Clin Pediatr 1978;17:113-118

6. Christie CDC, Baltimore RS: Pertussis in neonates. Am J Dis Child 1989;143:1199-1202

7. Brouwer AFJ, Gils JF van, Brand PLP, Graaf JH de: Perinatale pertussis: van moeder naar kind. Ned Tijdschr Geneesk 2001;145:2257-2259

8. Hoppe JE: Neonatal pertussis. Pediatr Infect Dis J 2000;19:244-247

9. MacLean DW, Calder MA: Pertussis in pregnancy. Scott Med J 1981;26:250-253

10. Granström G, Granström M, Sterner G: Whooping cough in late pregnancy. Scand J Infect Dis 1990; suppl. 71:27-29

11. Smith C, Vyas H: Early infantile pertussis; increasingly prevalent and potentially fatal. Eur J Pediatr 2000;159:898-900

12. Kurt TL, Yeager AS, Guenette S, Dunlop S: Spread of pertussis by hospital staff. JAMA 1972;221:264-267

13. Vincent JM, Wack RP, Person DA, Bass JW: Pertussis as the cause of recurrent bradycardia in a young infant. Pediatr Infect Dis J 1991;10:340-342

14. Aujard Y, Bedu A, Baumann C, Bingen E: La coqueluche du nouveau-né. Méd Mal Infect 1995;25:1268-1271

15. Hoppe JE: Keuchhusten bei Schwangeren und Neugeboren. Z Geburtsh Neonatol 1996;200:241-244

16. Altemeier WA, Ayoub EM: Erythromycin prophylaxis for pertussis. Pediatr 59:623-625,1977

17. Versteegh FGA, Schellekens JFP, Nagelkerke AF, Roord JJ: Laboratory-confirmed reinfections with *Bordetella pertussis*. Acta Paediatr 2002;91:95-97

18. Wirsing von König CH, Postels-Multani S, Bock HL, Schmitt HJ: Pertussis in adults: frequency of transmission after household exposure. Lancet 1995;346:1326-1329

19. Deeks S, De Serres G, Boulanne N et al: Failure of physicians to consider the diagnosis of pertussis in children. Clin Infect Dis 1999;28:840-846

20. Long SS, Welkon CJ, Clark JL: Widespread silent transmission of pertussis in families: antibody

correlates of infection and symptomatology. J Infect Dis 1990;161:480-486

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

22. Laing JS, Hay M: Whooping-cough: its prevalence and mortality in Aberdeen. Public Health 1902;14:584-598

23. Granström G, Sterner G, Nord CE, Granström M: Use of erythromycin to prevent pertussis in newborns of mothers with pertussis. J Infect Dis1987;155:1210-1214

24. Melker HE de, Versteegh FGA, Heijden OG van der, Teunis PFM, Schellekens JFP, Kretzschmar MEE: Incidence of reported symptomatic infections versus incidence of all infections with *Bordetella pertussis* (submitted)

25. Melker HE de, Versteegh FGA, Conyn-van Spaendonck MAE et al: Specificity and sensitivity of high levels of Immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis.* J Clin Microbiol 2000;38:800-806

26. Arciniega JL, Hewlett EL, Edwards KM, Burns DL: Antibodies to *Bordetella pertussis* adenylate cyclase toxin in neonatal and maternal sera. FEMS Immunol Med Microbiol 1993;6:325-330

27. Oda M, Cowell JL, Burstyn DG, Thaib S, Manclark CR: Antibodies to *Bordetella pertussis* in human colostrum and their protective activity against aerosol infection of mice. Infect Immun 1985;47:441-445

28. Englund JA, Anderson EL, Reed GF et al: The effect of maternal antibody on the serologic response and the incidence of adverse reactions after primary immunization with acellular and whole-cell pertussis vaccines combined with diphtheria and tetanus toxoids. Pediatrics 1995;96:580-584

29. Glezen WP, Alpers M: Maternal immunization. Clin Infect Dis. 1999;28:219-224

30. Oda M, Izumiya K, Sato Y, Hirayama M: Transplacental and transcolostral immunity to pertussis in a mouse model using acellular pertussis vaccine. J Infect Dis 1983;148:138-145

31. Cohen P, Scadron SJ: The placental transmission of protective antibodies against whooping cough. JAMA 1943;121:656-662

32. Beiter A, Lewis K, Pineda EF, Cherry JD: Unrecognized maternal peripartum pertussis with subsequent fatal neonatal pertussis. Obstetr Gynecol 1993;82:691-693

33. Aoyama T, Ide Y, Watanabe J, Takeuchi Y, Imaizumi A: Respiratory failure caused by dual infection with *Bordetella pertussis* and respiratory syncytial virus. Acta Paediat Japon 1996;38:282-285

34. Wirsing von König CH, Rott H, Bogaerts H, Schmitt HJ: A serological study of organisms possibly associated with pertussis-like coughing. Pediatr Infect Dis J 1998;17:645-649

35. Stott NCH, Davis RH: Pertussis vaccination and pseudo whooping cough. Br Med J 1981;282:1871

36. Schellekens JFP, Melker HE de: Laboratoriumdiagnostiek van infecties met Bordetella spp. bij patiënt en populatie. Ned Tijdschr Med Microbiol 2000;8:112-116

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

38. Bruss JB, Malley R, Halperin S et al: Treatment of severe pertussis: a study of the safety and pharmacology of intravenous pertussis immunoglobulin. Pediatr Infect Dis J 1999;18:505-511

39. Versteegh FGA, Roord JJ: Therapie van en handelwijze bij kinkhoestpatiënten. Ned Tijdschr Med Microbiol 2001;9:20-21

40. Weber DJ, Rutala WA: Pertussis: a continuing hazard for healthcare facilities. Infect Control Hosp Epidemiol 2001;22:736-740

41. Forsyth KD, Campins-Marti M, Caro J, Cherry JD, Greenberg D, Guiso N, Heininger U, Schellekens J, Tan T, Wirsing von König CH, Plotkin S; Global Pertussis Initiative: New pertussis vaccination strategies beyond infancy: recommendations by the Global Pertussis Initiative. Clin Infect Dis 2004;39:1802-1809. Epub 2004 Dec 15

42. Hethcote HW: Simulations of pertussis epidemiology in the United States: effects of adult booster vaccinations. Math Biosci 1999;158:47-73

43. Van Rie A, Hethcote HW: Adolescent and adult pertussis vaccination: computer simulations of five new strategies. Vaccine 2004;22:3154-3165

44. Riffelmann M, Koesters K, Saemann-Ischenko G, Schmitt HJ, Wirsing von König CH: Antibodies to pertussis antigens in pediatric health care workers. Pediatr Infect Dis J 2002;21:381-383

45. Linnemann CC Jr, Ramundo N, Perlstein PH, Minton SD, Englender GS: Use of pertussis vaccine in an epidemic involving hospital staff. Lancet 1975;2:540-543

Chapter 8

Serologic evidence of possible mixed infections with *Bordetella pertussis* and other respiratory pathogens

F.G.A. Versteegh [1], E.A.N.M. Mooi-Kokenberg [2], J.F.P. Schellekens [3], J.J. Roord [4]

1: Groene Hart Ziekenhuis, Department of Pediatrics, Gouda, the Netherlands

2: Groene Hart Ziekenhuis, Department of Medical Microbiology and Infection Control, Gouda, the Netherlands

3: National Institute of Public Health and the Environment, Diagnostic Laboratory of Infectious Diseases and Perinatal Screening, Bilthoven, the Netherlands

4: Vrije Universiteit Medical Center, Department of Pediatrics, Amsterdam, the Netherlands

Abstract

Background: In pertussis-like respiratory infections, once pertussis has been laboratory confirmed, other potential causative pathogens will seldom be looked for. Probably most mixed infections are found accidentally and since these mixed infections might cause a more severe disease we performed a retrospective study of their incidence.

Patients and methods: We selected from 2 groups of patients with serologically confirmed *Bordetella (B.) pertussis* infection those in whom serology for other respiratory pathogens had also been performed. Group 1 consisted of 50 pertussis patients with 51 episodes of *B.pertussis* infection selected from 100 patients with serologically confirmed pertussis. They participated in a long term follow up after a *B.pertussis* infection. In group 2, 31 pertussis patients were selected from 98 consecutive patients with positive pertussis serology from one routine practice.

Results: In 23 of 82 pertussis infections (28%) serological evidence of one (n=21) or two (n=2) additional infections were demonstrated. These involved para-influenza virus (n=6), RSV (n=6), *Mycoplasma pneumoniae* (n=5), adenovirus (n=4), influenza A virus (n=3) and influenza B virus (n=1).

Conclusions: We conclude that in patients with *B.pertussis* infection, co-infection with another respiratory pathogen is often present.

Introduction

Because both viral and bacterial infections are common during early childhood, dual infections are not unexpected. However, clinical manifestation of such combined infections may be difficult to interpret (1) despite an increase in knowledge concerning microbial agents associated with acute respiratory infections of childhood (2,3). For many years there has been an interest in the dynamic interaction between pathogens and the influence on disease interference (1,4-12). Studies on *Bordetella (B.) pertussis* infection often associated adenoviruses and other respiratory viruses with the pertussis-like syndrome in the absence of clear evidence of *B.pertussis* infection (5,13-15). The presence of more than one respiratory pathogen in an ill infant may have important diagnostic, therapeutic, prognostic, epidemiological and infection control consequences (6). Despite the relatively high frequency with which both viral and *B.pertussis* infection may occur in the community, the occurrence of a combination of two or more microbiological agents (or mixed infection) is underestimated (6), both in infants and in adults. We retrospectively investigated the incidence of mixed infections with other respiratory pathogens in pertussis patients.

Materials and Methods

Patients

We considered two groups of patients, both with serologically confirmed *B.pertussis* infection and further selected those patients in whom serology for other respiratory pathogens had also been done.

The first group consisted of 100 patients taken from one clinical practice between 1992 and 1999, except 1998 (see below). They were all evaluated for prolonged coughing and had clinically documented (≥2 weeks paroxysmal cough) with serologically confirmed pertussis.

Group 2 consisted of 98 consecutive patients with serologically confirmed pertussis from one clinic (Groene Hart Hospital Gouda) in 1998. In that year the in- and outpatients physicians of the hospital tested a total of 416 patients for *B.pertussis* infection on account of prolonged coughing,

In these two groups we looked retrospectively whether serological data on other respiratory pathogens were available from the same sera positive for *B.pertussis* infection.

The study was approved by the Medical Ethical Committee of our hospital.

Serology for pertussis

In group 1 the clinical diagnosis of pertussis was confirmed by a significant (i.e., ≥ 4-fold) increase of IgG antibodies to pertussis toxin (IgG-PT) in paired sera to a level of at least 20 U/ml or a high IgG-PT concentration in a single serum, i.e. above a defined diagnostic cutoff of 100 U/ml, as measured by the in-house IgG-PT ELISA (16) of the National Institute of Public Health and the Environment (Dutch acronym: RIVM), Bilthoven, The Netherlands.

In group 2 the clinical suspicion of pertussis was confirmed by a significant increase of IgG antibodies to a mixture of pertussis toxin and filamentous haemagglutinin (IgG-PT/FHA) in paired sera. The criterion being either the value [OD-ratio*10] in second serum minus value in first serum ≥ +8) or a high IgG-PT/FHA in a single serum, i.e. above a defined diagnostic cutoff (criterion: value [OD-ratio*10] in first and/or second serum ≥ 28), as measured in a commercial IgG-PT/FHA ELISA (Virotech, Germany) (17).

Serology for other respiratory pathogens

Sera of patients in group 1 and 2 had also been tested with the complement fixation method (Serion Immunodiagnostics GmbH, Germany) and in some cases an indirect IgM immunofluorescent assay (Serion Immunodiagnostics GmbH, Germany) for antibodies against respiratory syncytial virus (RSV), influenza virus A and B, adenovirus, para-influenza virus 1, 2 and 3, *Mycoplasma (M.) pneumoniae*, Chlamydia species and *Coxiella (C.) burnetii*. Two point serology was considered as proof of a recent infection when there was a

significant i.e. fourfold increase or decrease in titer. In one point serology a titer ≥128 was always considered as indicative of a recent infection. A titer of ≥ 64 was considered suspect, except in *Coxiella burnetii* (≥ 4 suspect) and in *Chlamydia* (≥8 suspect)(18). An indirect IgM immunofluorescent assay (Serion Immunodiagnostics GmbH, Germany) was performed when the complement fixation method was suspect or not conclusive. A positive IgM was also considered as proof of a recent infection (18). Between para-influenza virus type 1, 2 and 3 there are serological cross reactions therefore they are reported together.

Results

First we compared characteristics of the two groups of pertussis patients. The median age of the patients in group 1 was 4.1 year (IQR 1.7 – 7.2; range 4 weeks to 12 years) and in group 2 this was 4.9 years (IQR 2.8 – 8.0, range 6 weeks tot 15 years)(p=0.20); the median disease duration at presentation was 30 days (IQR 11 – 49) days and 30 days (IQR 16 – 38) respectively (p=0.92). In group 1 54% was male and in group 2 61% (p=0.82). The proportion completely vaccinated against pertussis (≥ 3 immunizations with pertussis whole cell vaccine in the first year of life) was 78% and 74%, respectively.

From 50 of the 100 pertussis patients in group 1 serology data for other respiratory pathogens were available. One of the selected patients suffered from 2 episodes of *B.pertussis* infection (19), with an interval of 6 years and 7 months. Thus in group 1 there were 51 episodes of *B.pertussis* infection.

In group 2 from 31 of the 98 pertussis patients serology data for other respiratory pathogens was found. In group 1, sera of 14 of 51 (27%) patients showed signs of a mixed infection. In 2 of these sera, two other respiratory pathogens besides *B.pertussis*, were detected. In group 2, one other respiratory pathogen was detected in 9 sera of 31 patients (29%). Since no statistical significant differences were found in these parameters we decided to combine the groups for further analysis.

The age-distribution of all pertussis patients, with differentiation of those with and those without mixed infection, is shown in Figure 1. In 23 out of 82 pertussis episodes (28%) serological evidence for mixed infection was found. In 21 episodes one other respiratory pathogen was found (26%) with involvement of para-influenza virus (n=5), RSV (n=5), *M. pneumoniae* (n=4), adenovirus (n=4) and influenza A virus (n=3). In 2 episodes two other respiratory pathogens were found (2%), with in one case para-influenza virus and *M. pneumoniae* and in the other case influenza B virus and RSV (Fig 1). As shown in table 1 the median ages in patients with mixed (n=23) and patients with non-mixed infection (n=59) were 5.0 (IQR 3.0 – 8.1) years and 4.2 (IQR 1.8 – 7.3) years respectively (p=0.38); the median duration of disease at presentation was 17 days (IQR 13 – 36) and 31 days (IQR 20 – 46), respectively (p=0.09). The proportion completely vaccinated was respectively 78% and 75% (p = 0.84).
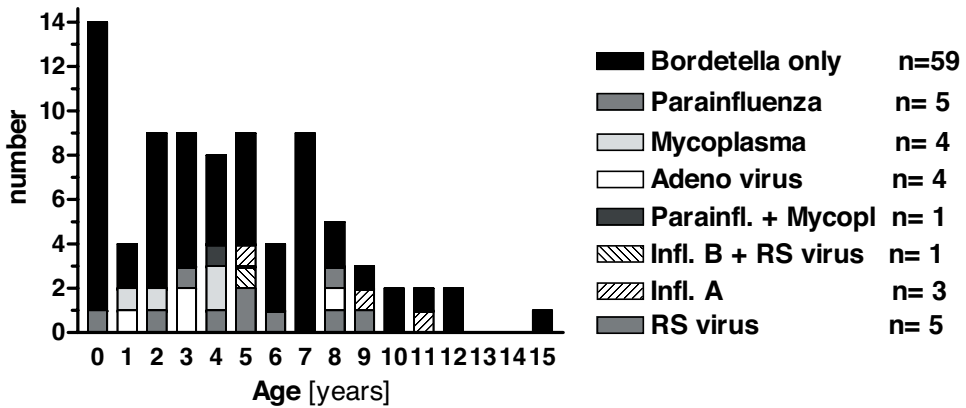
Infections N=82

Fig 1: All patients in whom serology was performed for both *Bordetella pertussis* and other respiratory pathogens, distributed by age. In black those with *Bordetella pertussis* infection only (n=59), the others (n=23) with mixed infection of *Bordetella pertussis* and one or two other respiratory pathogens.

In 11 of the 23 patients with mixed infection (48%), both pertussis serology and serology for other respiratory pathogens showed high (diagnostic) values in the first serum-sample. The median time these sera were obtained was 39 days after the first day of the disease (range 7-92 days).

Table 1: Data on patients with mixed or non-mixed infection

	Mixed Infection (N=23)	Non-mixed Infection (N=59)	p-value
Age (years)	5.0(3.0-8.1)*	4.2(1.8-7.3)*	0.38
Age range (years)	0.7 – 11.1	0.1 – 15.3	
Proportion < 1 year of age	4%	22%	0.10
Proportion completely vaccinated	78%	75%	0.84
Vaccinated	83%	82%	1.0
Disease duration at presentation	17(13-36)*	31(20-46)*	0.09

*= median and interquartile range.
Disease duration = number of days between first day of disease and first presentation to the clinic

In 2 of the patients (9%) pertussis serology was already high in the first sample while serology for other respiratory pathogens showed a significant increase in paired sera. The first sera were obtained 9 and 22 days after the onset of disease, the second after 31 and 40 days respectively. In 6 patients (26%) pertussis antibodies in paired sera had significantly increased, while the first serum already contained high (diagnostic) antibody titers against another respiratory pathogen (first serum: median 11 days (range 6-22), second serum: median 41,5 days (range

29-54)). Finally, in 4 of the 23 patients (17%) there was a significant increase in paired sera for antibodies against both, *B.pertussis* and one other pathogen (first serum: median 14 days (range 8-29), second serum: median 41 days (range 29-55)).

There were 3 patients younger than 2 years, in whom vaccination might have influenced *B.pertussis* serology. In one, 1.6 years, there was an increase in IgG-PT from 1 to 500 U/ml, another, 1.7 years, had approximately 3 months after the first day of the disease and 9 months after his last vaccination a titer of 500 U/ml (both RIVM). The third, aged 0.7 years, about 4 months after his third vaccination and with a concomitant RSV infection, had a titer of 33 (Virotech).

DISCUSSION

In this retrospective study we evaluated two groups of patients with a serological confirmed *B.pertussis* infection in whom also serology against other respiratory pathogens was performed. The first group had a cough ≥ 2 weeks, consistent with clinical pertussis. And the second group at least was suspected to have pertussis. That we don't have more detailed clinical data on these patients limits the use of this study a little, especially regarding disease severity with or without mixed infection.

We found that the incidence of mixed infection in both groups was similar (27 and 29%). The specific property of the first group of pertussis-patients was that after the pertussis episode they had returned to the same physician (F.V.) for other reasons, mostly because of asthma and therefore could participate in a long-term follow-up study of antibody-titers after pertussis (20). This may have introduced a selection-bias with respect to chance of mixed respiratory infection. However, the second group, with 29% mixed infection, consisted of 31 of 98 consecutive pertussis-patients (32%) testing positive in pertussis-serology at one clinic. It can therefore be inferred that the incidence of mixed respiratory infection in all pertussis patients from group 2 was at least 9% (0.32x29%) and probably higher. By our knowledge there was no mechanism associated with decisions whether or not to do serology for other respiratory infections. We consider therefore both groups to be a random sample from the total of patients and evaluated them together because there were no significant differences between the two.

Data in the literature vary greatly in their percentages of mixed infections, between 8 and 44% (7,8,21-23). This is possibly due to differences in studied groups, used methods and different respiratory pathogens looked for (table 2).

As might be expected, RSV, para-influenza virus, *M. pneumoniae* and adenovirus were the most encountered agents (24,25) (Fig1). The role of adenovirus infection in association with *B.pertussis* has not been clearly defined (26). Nelson et al reported their data suggesting that many adenoviruses isolated from their patients were latent viruses that had been activated

during the infection with *B.pertussis* rather than having been acquired exogenously (5). Discovery of new pathogens like the human meta-pneumovirus or a new human coronavirus (2,3) may also give rise to detection of more mixed respiratory infections.

Table 2: Data on mixed infection in different studies (7,8,21-23)

study	No patients studied for mixed infection	*B.pertussis* infection	*B.pertussis* and mixed infections(%)	comment
Moshal (7)	25	25	5 (20%)	children<6months
Jackson (8)	319	47	17 (36%)	students
MacLean (21)	43	4	1 (25%)	pregnant women
Smith (22)	9	9	4 (44%)	babies, 6 died, 3 with mixed infection
Ferrer (23)	905	114	9 (7,9%)	children

The frequency of *B.pertussis* infection measured by serology only might be influenced by recent vaccination in children < 2 years. But Nagel showed that vaccination with the Dutch whole cell vaccine induced only rather low IgG-PT levels (16). But other vaccines might induce higher IgG-PT levels since the response to PT varies between different whole-cell vaccines and acellular vaccines (20). Of the 3 patients <2 years, the 2 oldest had proof of recent infection. In the youngest *B.pertussis* serology might be influenced by recent vaccination.

The mentioned interpretation-criteria of the IgG-PT ELISA have been shown to yield a sensitivity in paired sera of PCR or culture confirmed pertussis patients (n=89) of 94% (in 76% of the patients a value ≥ of 100 E/ml in first and/or second serum was found). The specificity, in population sera (n=7759), was 99% (20) and, in paired sera of patients with respiratory disease of other etiology (n=59) 95% (in 3 of 59 patients a value ≥ 100 U/ml was found in first or second serum; significant dynamics were absent) (17).

In a previous investigation of the performance of this commercial ELISA in paired sera of a group of patients with culture- or PCR-confirmed pertussis (n=56) and a control group with respiratory disease of other etiology (n=59), the mentioned criteria for significant dynamics in paired sera and diagnostic values in single sera have been shown to yield a sensitivity of 91% (87% had a value ≥ 28 in first and/or second serum). The specificity was 95% (3 of 59 control-patients had a value ≥ 28 in first and/or second serum; significant dynamics were absent) (17).

The IgG-PT/FHA ELISA (Virotech, Germany) is not specific for *B.pertussis* but may also indicate a *B.parapertussis* infection. In this study there is no possibility to discriminate between these 2 agents.

From our data it is difficult to conclude whether or not mixed infections were simultaneous

or consecutive. In 48% of the patients with mixed infection, the finding of already high diagnostic titers in a first serum against two or more pathogens, cannot differentiate whether the infections were concurrent or consecutive. An increase of antibodies against two or more pathogens in two consecutive sera occurred in 17% of the patients with mixed infection. This strongly supports the view that the infections have occurred at the same time. A high titer in the first serum for one agent and an increase for the other in a second serum favors a consecutive infection. This occurred in 35% of the patients with mixed infection.

Although mixed infections of *B.pertussis* with another respiratory pathogen seem to be more frequent than expected, their clinical relevance is still being discussed. In some studies, mixed or super-infection with *B.pertussis* is considered to be a major contributing factor to complications (9,22). Aoyama et al (9) presented two cases illustrating that RS-viral infection could cause pneumonia in pertussis patients that was severe enough to result in respiratory failure. In contrast, Moshal et al state that there are no specific clinical features that distinguish patients with only *B.pertussis* infection from those with a mixed infection with other respiratory viruses (7). Nelson et al (11) also could not find laboratory or clinical differences between *B.pertussis*, RSV or mixed infections in hospitalized children. One might hypothesize that patients with mixed infections are more likely to seek medical help because of more severe disease and doctors are possibly more likely to perform more extensive investigations when patients are seriously ill. We have however no sufficient data regarding the clinical status of all the patients in our study. The median duration of disease at presentation was shorter in patients with mixed infection (17 days) than in patients without mixed infection (31 days). Although this difference is not statistically significant (p=0.09), it may be taken as some support for the hypothesis that mixed infections are associated with more severe symptoms, prompting earlier presentation to the physician. It is also possible that mixed infections started with a respiratory viral infection which might be the reason why they were studied earlier.

The diversity of the other respiratory agents makes cross-reacting antibody responses unlikely but does not rule out nonspecific polyclonal responses (8).

Theoretically, the immature immune system of infants and lack of previous exposure to respiratory viruses could increase susceptibility to simultaneous infection with two or more respiratory pathogens. In a study where double respiratory viral infections were compared to patients with single respiratory viral infections a greater percentage of the first group are under the age of one year. However, in our patients the incidence of mixed infection in < 1 year olds was not higher than in other age-groups. Thus, it is unclear whether younger patients are more often infected with mixed respiratory infection or suffer more (10,23).

Failure to recognize one of the involved pathogens in hospitalized children may result in incorrect management. It also puts other patients, especially the immunocompromised and those with underlying heart or pulmonary disease, at risk of contracting potentially fatal

nosocomial infections. This is particularly important in winter when the hospitals are full and children are often cohorted according to the pathogen identified (e.g. respiratory syncytial virus). In addition patients are kept in isolation according to the pathogen causing the illness. It is imaginable that contracting a second agent might influence the course of the disease (duration, severity). Whether the mixed infections are really mixed or consecutive might, in this regard, not be very important.

Persistent cough in infants is a medical problem with different therapeutic options depending on etiology. Increased knowledge about the incidence of various possible causative micro-organisms is therefore important. Until the present day it remains unclear what the precise relationship is between pathogens in mixed infection.

In conclusion we think that mixed respiratory infections are an underestimated problem in children. Often when one agent is discovered the search for others is stopped. What is not looked for, will not be found. Infection with more than one microbiological agent may influence the clinical course of the disease (duration, severity). In this retrospective study we did not analyze the clinical impact of mixed infections of *B.pertussis* with other respiratory pathogens. Only a prospective study of children with prolonged cough with both *B.pertussis* and simultaneous identification of other respiratory agents could resolve this question. In view of the possible clinical importance of mixed infections, the recent progress in rapid viral diagnoses and the potential of new anti-bacterial and antiviral drugs, the possibility of dual infection should be investigated more often.

Acknowledgements

References

1. Dagan R, Hall CB, Menegus MA: Atypical bacterial infections explained by a concomitant virus infection. Pediatrics 1985;76:411-4

2. Hoogen BG van den, de Jong JC, Groen J, Kuiken T, Groot R de, Fouchier RA, Osterhaus AD: A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001;7:719-24

3. Hoek L van der, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC, Wertheim-Van Dillen PME, Kaandorp J, Spaargaren J, Berkhout B: Identification of a new human coronavirus. Nat Med. 2004;10:368-73

4. Rohani P, Green CJ, Mantilla-Beniers NB, Grenfell BT: Ecological interference between fatal diseases. Nature 2003;422:885-8

5. Nelson KE, Gavitt F, Batt MD, Kallick CA, Reddi KT, Levin S: The role of adenoviruses in the pertussis syndrome. J Pediatr 1975;86:335-41

6. Tristram DA, Miller RW, McMillan JA, Weiner LB: Simultaneous infection with respiratory syncytial virus and other respiratory pathogens, Am J Dis Child 1988;142:834-6

7. Moshal KL, Hodinka RL, McGowan KL: Concomitant viral and *Bordetella pertussis* infections in infants. Pediatr Infect Dis J 1998;17:353-4

8. Jackson LA, Cherry JD, Wang S-P, Grayston JT: Frequency of serological evidence of Bordetella infections and mixed infections with other respiratory pathogens in university students with cough illnesses. Clin Infect Dis 2000;31:3-6

9. Aoyama T, Ide Y, Watanabe J, Takeuchi Y, Imaizumi: A. Respiratory failure caused by dual infection with *Bordetella pertussis* and respiratory syncytial virus. Acta Paediatr Jpn 1996;38:282-5

10. Drews AL, Atmar RL, Glezen WP, Baxter BD, Piedra PA, Greenberg SB: Dual respiratory virus infections. Clin Infect Dis 1997;25:1421-9

11. Nelson WL, Hopkins RS, Roe MH, Glode MP: Simultaneous infection with *Bordetella pertussis* and respiratory syncytial virus in hospitalized children. Pediatr Infect Dis 1986;5:540-4

12. Biedert P: Beobachtungen über Disposition zu Keuchhusten, Masern und Scharlach, sowie über Prophylaxe derselben. Jahrb.Kinderheilk. 1886;24:93-104

13. Connor JD: Evidence for an etiologic role of adenoviral infection in pertussis syndrome. N Engl J Med 1970;283:390-4

14. Sturdy PM, Court SD, Gardner PS: Viruses and whooping-cough. Lancet 1971;2:978-9

15. Wirsing von König CH, Rott H, Bogaerts H, Schmitt HJ: A serological study of organisms possibly associated with pertussis-like coughing. Pediatr Infect Dis J 1998;17:645-9

16. Nagel J, de Graaf S, Schijf-Evers D: Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LPF antibody levels. Dev Biol Stand 1985;61:325-30

17. Meijer BC, Peeters MF, Schellekens JFP: Serodiagnostiek van kinkhoest met commerciële Elisa's

Voorjaarsdagen NVMM, Veldhoven 20-21 april 1999. Ned Tijdsch Med Microbiol 1999;7 suppl: S15

18. Herrmann KL: Antibody detection. In: Lennette EH, Halonen P, Murphy FA (eds.): Laboratory diagnosis of Infectious diseases. Principles and practice. Vol. II. Berlin: Springer Verlag, 1988:76-101

19. Versteegh FGA, Schellekens JFP, Nagelkerke AF, Roord JJ: Laboratory-confirmed reinfections with *Bordetella pertussis*. Acta Paediatr 2002;91:95-7

20. Melker HE de, Versteegh FGA, Conyn-Van Spaendonck MA, Elvers LH, Berbers GA, van der Zee A, Schellekens JFP: Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*, J Clin Microbiol 2000;38:800-6

21. MacLean DW, Calder MA: Pertussis in pregnancy. Scott Med J 1981;26: 250-3

22. Smith C, Vyas: H. Early infantile pertussis; increasingly prevalent and potentially fatal. Eur J Pediatr 2000;159:898-900

23. Ferrer A, Calicó I, Manresa JM, Andreu A, Moraga F, Valle I: Microorganismos aislados en casos de síndrome pertusoide. Enferm Infec Microbiol Clin 2000;18:433-8

24. Wesley AG, Windsor IM: Viral infections in clinical pertussis. S Afr Med J 1983;64:577-8

25. Cherry JD, Heiniger U: Pertussis and other Bordetella infections In: Feigin RD, Cherry JD, eds. Textbook of pediatric infectious diseases. 4th edition vol.2. Philadelphia: Saunders, 1998:1423-40

26. Keller MA, Aftandelians R, Connor JD: Etiology of pertussis syndrome. Pediatrics 1980;66:50-5

Chapter 9

Pathogens in community acquired coughing in children: a prospective cohort study

F.G.A. Versteegh [1], G.J. Weverling [2], M.F. Peeters [3], B. Wilbrink [4], M.T.M. Veenstra-van Schie [1], J.M. van Leeuwen-Gerritsen [1], E.A.N.M. Mooi-Kokenberg [5], J.F.P. Schellekens [4], J.J. Roord [6]

1. Groene Hart Ziekenhuis, Department of Pediatrics, Gouda, The Netherlands
2. Academic Medical Center, Department of Clinical Epidemiology and Biostatistics, University of Amsterdam, Amsterdam, The Netherlands
3. St.Elisabeth Hospital, Department of Medical Microbiology, Tilburg, The Netherlands
4. National Institute of Public Health and the Environment, Diagnostic Laboratory of Infectious Diseases and Perinatal Screening, Bilthoven, The Netherlands
5. Groene Hart Ziekenhuis, Department of Medical Microbiology and Infection Control, Gouda, The Netherlands
6. Free University Medical Center, Department of Pediatrics, Amsterdam, The Netherlands.

Abstract

To investigate in children with prolonged coughing the frequency of different respiratory pathogens, the rate of mixed infections and possible differences in severity of disease between single and mixed infections, we performed a 2 years prospective study.

Children from 0-18 years with coughing lasting 1 to 6 weeks were included. Sera of 135 children (with 136 episode of prolonged coughing) were tested for antibodies against different viruses and bacteria. Swabs were taken for culture and PCR on different viral and bacterial pathogens.

One or more pathogens were found in 91 patients (67%). In 49 (36%) one infectious agent was found, in 35 (26%) 2 and in 7 (5%) more than 2. The most frequent pathogens encountered were rhinovirus (43 patients-32%), *Bordetella pertussis* (23 patients-17%) and respiratory syncytial virus (15 patients-11%).

The most frequent encountered mixed infection consisted of a combination of *Bordetella pertussis* and rhinovirus (n=14-10%).

No significant differences were observed regarding clinical symptoms between patients without pathogens and those in whom one or more pathogens were found, except that patients with a mixed infection were significantly older. There is a strong seasonal influence in the number of infections but not in the number of mixed infections.

In children with prolonged coughing there is a high frequency of mixed infections occurring during the whole year regardless of the season. Mixed infection however does not cause increased disease severity. Although patients with mixed infection were significantly older, no clinical symptoms were found that discriminate for specific pathogens.

Introduction

Prolonged coughing is a frequent symptom in children and often is associated with respiratory infection [1]. Although prolonged coughing is a prominent feature in *Bordetella (B.) pertussis* infection, infections with other respiratory pathogens may cause prolonged coughing as well [2]. Mixed infections with a combination of 2 or more pathogens do occur. However data on clinical manifestations of such combined infections may be difficult to interpret and it often concerns retrospective studies with different results [2, 3-5]. It has been suggested that mixed infections may cause more severe illness, especially in younger children [3, 4, 6]. In an earlier retrospective observational study (data not published) among 81 children with serologically proven *B.pertussis* infection we found in 28% of the patients evidence for concomitant infections with other respiratory pathogens. It is known that many respiratory pathogens may cause prolonged coughing in children, however prolonged coughing is not always caused by

a respiratory infection [1, 7, 8].

We conducted this study to investigate the role of different respiratory pathogens in prolonged coughing in children and the frequency of possible mixed infections. We also studied whether patients in whom one or more pathogens were detected suffered from more severe disease than patients without pathogens.

History

Patients:

All patients between 0 and 18 years, referred by general practitioners from September 2001 till September 2003 to the outpatient clinic of the department of Pediatrics of the Groene Hart hospital, a 500-bed general hospital in Gouda, The Netherlands, with the complaint of coughing one week or more and not longer than 6 weeks at the first visit were asked to participate in the study. Disease duration was defined as the time between onset of symptoms and first visit to the hospital. Prolonged, chronic or persistent cough is defined differently in various studies ranging from 5 days to more than one month [2, 7,9-12]. Our patient selection was based on a 1 to 6 weeks lasting cough since this is in agreement with most studies.

Patients with an aspiration of a foreign body or known to have cystic fibrosis were excluded. At the first visit blood samples were taken for erythrocyte sedimentation rate (ESR), C reactive protein (CRP), leukocyte count and differentiation and serology for respiratory pathogens (see below). In all patients oropharyngeal, nasal and nasopharyngeal swabs were also taken for culture and PCR on respiratory pathogens (see below). When disease duration at presentation at the first visit was less than 14 days, serology was repeated after two weeks except for *B.pertussis*. When serology and PCR at the first visit were negative for *B.pertussis*, serology was repeated 4 weeks after the first visit (6 weeks in children < 1 year). The patients or their parents were asked to complete a questionnaire about complaints, previous diseases and vaccination status. All gave their written informed consent to participate in the study. A follow-up telephone interview was conducted approximately 4 weeks after enrollment. The study was approved by the Medical Ethical Committee of our hospital. A healthy control group was not included in this study.

Pathogens:

In our observational study we sought to identify agents and studied their occurrence in relationship to prolonged coughing. Therefore we define pathogens as agents known to be able to cause disease. For sake of clarity we refer to these agents as pathogens whether or not they caused the illness of interest. In tables 2 and 3 these bacteria considered to reflect a carrier state are indicated in the light gray area.

Serology:

Serology for *B.pertussis* was considered positive by the finding of a significant, ≥ 4-fold increase of IgG antibodies to pertussis toxin (IgG-PT) in paired sera to a level of at least 20 U/ml or a high IgG-PT concentration in a single serum, i.e. above a defined diagnostic cutoff of 100 U/ml, as measured in the in-house IgG-PT ELISA of the National Institute of Public Health and the Environment (Dutch acronym: RIVM), Bilthoven, The Netherlands [13]. The mentioned interpretation-criteria of the IgG-PT ELISA have been shown to yield a sensitivity and specificity of respectively 90% and 97% [14]. All sera also were tested for antibodies against respiratory syncytial virus (RSV), influenza virus A and B, adenovirus, para-influenza virus 1, 2 and 3, *Mycoplasma* (*M.*) *pneumoniae*, Chlamydia species and *Coxiella* (C.) *burnetii* (by the Regionaal Medisch Microbiologisch Laboratorium, Rotterdam, The Netherlands) with the complement fixation method (Serion Immunodiagnostics GmbH, Germany). Two point serology against these pathogens was considered as proof of a recent infection when there was a significant i.e. fourfold or more increase or decrease in titer. In one point serology a titer ≥128 was always considered indicative of recent infection. A titer of ≥ 64 was considered suspicious, except in *C. burnetii* (≥ 4 suspicious) and in Chlamydia (≥8 suspicious) [15]. An indirect IgM immunofluorescent assay (Serion Immunodiagnostics GmbH, Germany) was performed when the complement fixation method was suspicious. A positive IgM together with suspicious serology was also considered as proof of a recent infection. Between para-influenza virus type 1, 2 and 3 there are serological cross reactions therefore they are reported together.

PCR:

The detection of *B.pertussis* and *B.parapertussis* was based on the polymerase chain reaction (PCR), using a MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche) and primer pairs based on insertion sequence elements IS481 and IS 1001. The final PCR product was analyzed by gel electrophoresis and by dot blot hybridization [16,17].

PCR for *Chlamydia* (*C.*) *pneumoniae, M.pneumoniae*, influenzavirus A and B, coronavirus 229E and OC43, RSV, human metapneumovirus (hMPV), rhinovirus and enterovirus were performed on a nasal and a throat swab. A negative control (virus transport medium) was included for every 4 clinical samples. A positive control containing each pathogen was included in each DNA and RNA extraction and PCR run. DNA was isolated with a proteinase-K/SDS extraction essentially as previously described [18]. RNA was isolated with a high pure RNA isolation kit (Roche Molecular Biochemicals) with the addition of poly A RNA as carrier according to the instructions from the manufacturer. An aliquot of the isolated DNA was used in a PCR detecting either *M.pneumoniae* [18] or *C.pneumoniae* [19]. An aliquot of the eluted RNA preparation was used for rhino/enterovirus [20], influenzavirus A [21] and B [22] and hMPV [23] were separately amplified in a single-tube RT-PCR. RSV [24] and coronavirus

OC43 and 229E [25] were amplified in a multiplex single-tube nested RT-PCR.

Culture for respiratory bacterial pathogens:
A oropharyngeal swab was inoculated onto a blood agar plate, a blood agar plate + oxolinicacid (10 mg/L) plate and a chocolate agar plate. Beta-hemolytic streptococci group A, *Haemophilus* (*H.*) *influenzae*, *Streptococcus* (*S.*) *pneumoniae* and *Moraxella* (*M.*) *catharralis* were considered potential pathogens. *Staphylococcus aureus*, *Neisseria meningitides*, β-hemolytic streptococci not group A and *Candida* (*C.*) *albicans* were also reported but not considered pathogenic. Only those cultures with predominant growth were considered positive [26]. Non pathogenic oropharyngeal flora (i.e viridans streptococci, difteroids, coagulase negative staphylococci etc) is not reported.

Statistical Analysis:
For the description of patient characteristics median and interquartile ranges were obtained. A statistical comparison was done between three groups of children: those in whom no pathogen could be found, children with only one pathogen and those with two or more pathogens. For this purpose chi-square test was used or, in case of continuous variables, analysis of variance (ANOVA) was used.

Results

During a 2 year period starting September 2001 a total of 152 patients were referred to our hospital for prolonged coughing; 11 patients refused to participate in the study. Five patients were not included due to protocol violation. The remaining 136 episodes were obtained from 135 patients since one patient had 2 episodes of coughing with an interval of 5.2 months which were considered as 2 independent events. To study seasonal influences patients were included in this prospective cohort study during a 2 year period. Most patients were included in the first year of the study.

Of these 136 patients 59% were male and the median age at inclusion was 2.7 years (interquartile range (IQR): 0.6 to 6.4 years) and the median duration of complaints at presentation was 14 days (IQR: 10 to 20 days). A total of 51 (38%) patients were hospitalized for treatment. Of the 136 children 124 (92%) were vaccinated against *B. pertussis* (table 1). Patient characteristics are summarized in table 1.

In 91 patients (67%) one or more pathogens were found. Of those, 49 had one pathogen (36%), 35 had 2 (26%), 6 patients had 3 pathogens (4%) and one had 4 pathogens (1%). In our study we found in 31% of the patients more than 1 respiratory pathogen (table 1). The presence of more than one pathogen in patients was found during the whole year and in all ages. In 33% no pathogen was found. The means of detection of the different pathogens are reported in table 2.

All respiratory pathogens found in this study were considered possible cause of the prolonged coughing complaints, although patients may be colonized by some of the pathogens found, without causing disease.

Table 1: Clinical data on all patients together and divided in patients without, one or more than one respiratory pathogen.

	All	Without pathogen	1 pathogen	More than 1 pathogen	p-value
N	136	45 (33%)	49 (36%)	42 (31%)	
Age *years*[A]	2.7 (0.6-6.4)	1.3 (0.6–5.8)	1.0 (0.4–5.0)	4.4 (1.8–9.0)	0.005
Male	80 (59%)	27 (60%)	31 (63%)	22 (52%)	0.56
Disease duration *days*[A]	14 (10 – 20)	15 (13–21)	14 (9–17)	14 (9–20)	0.33
Vaccinated [*]	124 (92%)	41 (91%)	44 (90%)	39 (95%)	0.64
Admission	51 (38%)	16(36%)	22 (45%)	13 (31%)	0.37
Length of stay *days*[A]	5 (3 – 7)	3.5 (2–6)	5 (3–7)	5 (4–7)	0.49
Antibiotics	68 (50%)	22 (49%)	23 (47%)	23 (55%)	0.75

[*] 1 missing information on vaccination status, [A] (median and interquartile range), § one missing culture.
Disease duration: time between first day of illness and first presentation in the hospital.
Vaccinated: received the regular vaccinations appropriate for their age.
Length of stay: days in hospital when admitted.

In these 91 patients the most frequently seen pathogens were rhinovirus in 43 patients (47%), *B.pertussis* in 23 patients (25%) and RSV in 15 patients (16%) (table 2). In the 42 patients with a mixed infection the most frequent combination was *B.pertussis* and rhinovirus (N=14) (table 3).

Rhinovirus, the most prevalent pathogen, was in 23 patients found together with one or more other pathogens. In 14 of the cases of rhinovirus this other pathogen was *B.pertussis*. *B.pertussis,* the second most frequent pathogen, was found in 18 patients together with another pathogen. In 14 patients this other pathogen was rhinovirus (table 3). RSV was found in 12 patients together with another pathogen (table 3).

Six patients had a combination of 3 pathogens: one with adenovirus, RSV and *B.parapertussis*, one with *B.pertussis*, *M.pneumoniae* and rhinovirus, one with *B.pertussis*, adenovirus and *H. parainfluenzae*, one with *M.pneumoniae*, β-hemolytic streptococci group B and rhinovirus, one with RSV, rhinovirus and *H.parainfluenzae* and the sixth had a combination of *M.pneumoniae*, *H.influenzae* no type b and β-hemolytic streptococci group G. The one patient with 4 pathogens had parainfluenzavirus, RSV, rhinovirus and *H.parainfluenzae.*

In this study 15 of the 27 positive bacterial cultures were found in patients with more than one pathogen (see table 3). There were 11 patients with a positive oropharyngeal culture and one

other pathogen. In only 1 of these 11 patients (with a CRP of 109 mg/L) we considered the bacterial infection of influence on the course of the disease. In the other 10 there was no sign of a bacterial infection. In 4 patients there were 2 or more other pathogens apart from the positive oropharyngeal swab (table 3).

Table 2: Number of respiratory pathogens detected in this study and means of detection.

Pathogen	Children with pathogen n=91 (67%)	Diagnostic test			
		Serology	PCR	Both	culture
Bordetella pertussis	23 (25%)	3	8	12	-
Bordetella parapertussis	1 (1%)	-	1	-	-
Influenzavirus A or B	5 (5%)	5	0	0	-
Adenovirus	6 (7%)	6	-	-	-
Mycoplasma pneumoniae	6 (7%)	4	1	1	-
Parainfluenzavirus	9 (10%)	9	-	-	-
Respiratory Syncytial virus	15 (16%)	14	1	0	-
Rhinovirus	43 (47%)	-	43	-	-
Humane metapneumovirus	1 (1%)	-	1	-	-
Enterovirus	4 (4%)	-	4	-	-
Chlamydia pneumoniae	1 (1%)	0	0	1	-
Streptococcus pneumoniae	1 (1%)	-	-	-	1
Haemophilus parainfluenzae	7 (8%)	-	-	-	7
Staphylococcus aureus	1 (1%)	-	-	-	1
Haemophilus influenzae no type b	12 (13%)	-	-	-	12
Haemophilus influenzae type b	1 (1%)	-	-	-	1
β-hemolytic streptococci group C	1 (1%)	-	-	-	1
β-hemolytic streptococci group G	1 (1%)	-	-	-	1
β-hemolytic streptococci group B	1 (1%)	-	-	-	1
Candida albicans	2 (2%)	-	-	-	2
total	141				

Between brackets the percentages of patients. This is more than 100% because in 42 patients 2 or more pathogens were found. Bacteria considered to reflect a carrier state are indicated in the light gray area.

-: not done

Thus, from the 42 (31%) patients with more than 1 pathogen, in 10 patients with a positive oropharyngeal culture and one other pathogen, it is difficult to say whether there is a mixed or a consecutive infection or whether the bacteria found represent a carrier state. Therefore these 10 were not considered as possible mixed infection. On the basis of serology, PCR and culture 21 from the remaining 32 (24%) patients were considered to have had a possible mixed

infection (14 of these with a positive PCR for 2 or 3 pathogens), 9 a consecutive infection and 2 both.

There are no significant differences between patients with and without pathogens regarding disease duration at presentation (i.e. time between first day of illness and first presentation at the hospital), disease severity (antibiotics, hospitalization, length of stay, coughing, other symptoms like headache, sore throat, or pain) or vaccination status (tables 1 and 4). Except that children with more than 1 pathogen were on average 3 years older compared to children without or with only 1 pathogen (p=0.005) (table 1). Although there was no significant difference in coughing during different parts of the day, there seemed a tendency for children with more than 1 pathogen to have more coughing (table 4). Also there were no significant

Table 3: Nature and number of respiratory pathogens in 42 patients with more than one pathogen found. Bacteria considered to reflect a carrier state are indicated in the light gray area.

	Bordetella pertussis	*Bordetella parapertussis*	Influenzavirus A or B	Adenovirus	*Mycoplasma pneumoniae*	Parainfluenzavirus	Respiratory Syncytial Virus	Rhinovirus
Bordetella pertussis								
Bordetella parapertussis								
Influenzavirus A or B								
Adenovirus	2□	1*	1					
Mycoplasma pneumoniae								
Parainfluenzavirus								
Respiratory Syncytial Virus	1		1			1		
Rhinovirus	14**			2		2%	2□	
Humane metapneumovirus								
Enterovirus	1		1				1	
Chlamydia pneumoniae								
Streptococcus pneumoniae						1		
Haemophilus parainfluenzae						1		1
Staphylococcus aureus								
Haemophilus influenzae no type b						1	3	1
Haemophilus influenzae type b					1			
β-hemolytic streptococci group C								
β-hemolytic streptococci group G					1@			
β-hemolytic streptococci group B								1**
Candida albicans							1	

In 6 patients 3 pathogens were found and in one patient 4 pathogens:

*	1 also respiratory syncytial virus	**	1 also *Mycoplasma pneumoniae*
□	1 also *Haemophilus parainfluenzae*	@	1 also *Haemophilus influenzae* no type b
%	1 also respiratory syncytial virus and *Haemophilus parainfluenzae*		

differences in coughing or other symptoms between the 3 possible pathogens most frequently found, except that patients with *B. pertussis* had less fever.

All our patients recovered in due time although in our study design it proved too difficult to record time to recovery accurately. Therefore there are no data on this subject available. Data on coughing in the family or other contaminators were not recorded.

Table 4: Number (percentage between brackets) of patients with coughing complaints and with different other symptoms. There are no significant differences. Also there are no significant differences between the 3 most frequent pathogens, except that there is less fever in *Bordetella pertussis*

	Without pathogen	1 pathogen	More than 1 pathogen		Bordetella pertussis	Rhinovirus	RS virus
All (n=135)*	45 (33%)	49 (36%)	41 (31%)*		23 (17%)	43 (32%)	15 (11%)
Coughing							
Morning (n=82)	25 (30%)	26 (32%)	31 (38%)		16 (20%)	30 (37%)	11 (13%)
Afternoon (n=70)	22 (31%)	21 (30%)	27 (39%)		17 (24%)	26 (37%)	9 (13%)
Evening (n=88)	25 (28%)	32 (36%)	31 (35%)		20 (23%)	31 (35%)	9 (10%)
Night (n=89)	25 (28%)	32 (36%)	32 (36%)		17 (19%)	29 (33%)	13 (15%)
Symptoms							
Fever (n=47)	18 (38%)	14 (30%)	15 (32%)		1 (2%)	13 (28%)	7 (15%)
Tachypnea (n=51)	16 (31%)	20 (39%)	15 (29%)		6 (12%)	15 (29%)	9 (18%)
Dyspnea (n=63)	22 (35%)	21 (33%)	20 (32%)		9 (14%)	14 (22%)	7 (11%)

* Missing data for 1 patient.

Discussion

In our 2-year observational study of 136 children with coughing lasting for 1 to 6 weeks, we investigated the occurrence of respiratory pathogens. The presence of one or more pathogens (mixed infection) was subsequently studied in relation to the occurrence of clinical symptoms and the treatment given. We found 91 patients (67%) who had 1 or more respiratory pathogens and overall a high incidence of infection with rhinovirus (32%), *B.pertussis* (17%) and RSV (11%). To our knowledge this is the first study where a broad spectrum of respiratory pathogens has been studied in a prospective manner in children selected because of their coughing pattern. Others have also reported rhinovirus to be a frequently found pathogen in respiratory disease in all ages [27-29] among which a long-term surveillance of acute respiratory infections in the

Netherlands [30]. There seems an age dependency in the incidence of rhinovirus infection, the incidence being lower in older people. Incidences ranged from 21% to 40% in children with infection but in one study [29] in children without nasal symptoms the incidence was also 20%. Although rhinovirus was considered earlier to cause mild upper airway disease, it becomes more and more clear, using more advanced diagnostic techniques, that it may play an important role in more severe respiratory infection. It is difficult to compare our results with the frequency of pathogens reported in other studies on respiratory infections among children since these were mainly retrospective studies focusing on specific pathogens or specific combinations of pathogens [2,4-6,8-10,30-44]. The incidence of pathogenic agents for respiratory infection in our study was 67%, which is comparable to some studies, with incidences ranging from 56% to 64% [2,9,33,40] but different from other studies reporting incidences from 80% to 90% [9,30,37]. The incidence is age dependent with a high frequency observed among the youngest patients and ranged from 80-90% in children up to 6 years to 40% in adults [30,31]. In our study, we did not find this relationship since the incidence among children from 1-4 years old was 63% and did not differ from the oldest children.

We observed a relatively high incidence of co-infection of rhinovirus and *B.pertussis* (10% of all patients). Among children infected with *B.pertussis* a co-infection with rhinovirus was found in 61% of the cases. The role of *B.pertussis* in rhinovirus infection is complicated since it has been reported that rhinovirus-induced changes in airway smooth muscle responsiveness in isolated rabbit and human airway smooth muscle (ASM) tissue and cultured ASM cells were largely prevented by pre-treating the tissues with pertussis toxin or with a monoclonal blocking antibody to intercellular adhesion molecule-1, the principal endogenous receptor for most rhinoviruses [45]. This might indicate that the co-infection of rhinovirus and *B.pertussis* is not as severe as one would expect. This hypothesis is confirmed by our observation where coughing and other clinical symptoms, did not differ significantly from patients with either rhinovirus or *B.pertussis* and the fact that despite 61% of co-infection of *B.pertussis* and rhinovirus, there was less fever in the *B.pertussis* group, as expected in *B.pertussis* infection. In all clinical parameters studied, like disease duration at presentation or treatment (table 1), coughing or disease symptoms no significant differences could be found between patients with and patients without demonstrable pathogen(s), except the fever, (table 4). Even when comparing children with rhinovirus, *B.pertussis* and RSV separately there were no statistical significant differences (table 4) nor in patients with a combined infection with rhinovirus and *B.pertussis*. This is in accordance with others [46]. Thus, decisions on treatment still will mainly be made on the physicians' clinical judgment.

In our study RSV infection might be underrepresented (11%), since many and especially young children with severe coughing were referred within one week after onset of cough and were excluded by definition.

We often encountered a negative PCR for RSV, influenzavirus and *M.pneumoniae* whereas a

positive serology was found for these agents (table 2). A false negative result is very unlikely, since positive controls were tested on a regular base throughout the study without false negative results. Therefore a short duration of pathogen replication might be the explanation.

In 33% of the patients in our study no pathogens or positive serology could be detected. This percentage is in agreement with other studies [2,9,31,44,46]. As yet unidentified pathogens may be associated with prolonged coughing in the serology-, culture- and PCR-negative group. The hMPV and human corona virus -NL63 are good examples of such pathogens that have been described recently [23,47]. Although hMPV is recently reported to be an important agent in respiratory disease especially in young children [48] we could neither confirm this in our study, nor say whether it influenced disease severity in mixed infection.

Unfortunately, we did not recruit asymptomatic children as a control group, because this was considered too distressing for children. However the occurrence of pathogens in subjects without coughing would provide data on the incidence of carrier rate without disease. Some researchers identified fewer viral agents in non-symptomatic controls compared to patients [32-35]. Gunnarsson et al. [31] found in patients with long standing cough more *S. pneumoniae*, *H.influenzae* and *M.catarrhalis* compared with healthy individuals of the same age. In the ARI-EL study, a case control study in general practitioner patients of all ages with acute respiratory infections in the Netherlands from October 2000 till October 2003, statistically significant fewer viruses were detected in controls (54% vs. 19%) [36]. Therefore it remains to be determined to what extent the presence of a pathogen is responsible for the coughing pattern.

Most patients with a positive culture for pathogenic bacteria will not have a bacterial respiratory infection. Since carriage rates for potential pathogenic bacteria in healthy young children may vary between 11 and 48% [31,49] it is difficult to establish what clinical influence these bacteria might have had in our patients. In tables 2 and 3 these bacteria considered to reflect a carrier state are indicated in the light gray area. In a logistic regression analysis, although not statistically significant, we found a greater association with coughing in the morning from pathogens diagnosed by PCR or serology as compared to diagnosed by the oropharyngeal bacterial culture method (odds ratio 2.0 vs. 0.8). Similar associations were found when coughing patterns on other parts of the day were analyzed. Therefore it could be argued that a positive bacterial oropharyngeal culture is not of much value in the search for respiratory pathogens causing prolonged cough.

We observed an occurrence of more than one pathogen in 31% of all included children. This might support the theory that the presence of one (viral) respiratory pathogen may predispose to a second or third (bacterial) infection [39,42,46]. The proportion of mixed infections reported by others varies largely 10 to 74% of the patients [9,33,34,38,39,46]. This might considerably be influenced by the period of observation. We have tried to exclude the seasonal influence by taking an observation period of 2 years. During this 2-year enrolment

of patients the seasonal influence varied significantly between the first and the second year. In the second study year a lower incidence of prolonged coughing was observed and there were fewer referrals, possibly due to the milder winter of 2002-2003 and the rather warm summer of 2003 in the Netherlands as compared to 2001-2002. Remarkable was therefore that the frequency of two or more pathogens during both years of the study in every season remained the same.

In previous studies it has been demonstrated mixed infections to be related with a more severe course of the disease [3-6,43,44,48]. In contrast, we found no evidence of a more severe illness in our patients with mixed infections (table 1). Apparently there is one dominant pathogen without a cumulative or synergistic effect of a second pathogen. Although it may be speculated that a viral infection might make the patient more vulnerable to a bacterial infection.

However, patients with more than 1 pathogen were significantly older than those with one or no pathogens. This might indicate that they were more ill than children of similar age with no detectable pathogen or just one. This is supported by the fact that there is a slight tendency for coughing complaints to be higher in patients with 2 or more pathogens (table 4).

Still it seems important to look for mixed infections [48] because when there is a high incidence of a respiratory infection (for instance RSV), sometimes patients are hospitalized in one room, increasing the risk of nosocomial infection. Although it seems mixed infections don't produce more severe disease, it is questionable if this is correct policy because of the possibility of a high incidence of infections with more than one pathogen. When they are consecutive, this may prolong admission.

In conclusion we found in children with prolonged coughing in 36% one pathogen and in 31% more than 1 pathogen. There is a strong seasonal influence on the number of cases but not on pathogens found nor on the percentage of multiple infections. Clinical data did not distinguish between pathogens, whether pathogens were found or not, or differences in treatment. In mixed or consecutive infection there was no increase in disease severity regarding symptoms and hospitalization. The only difference being that this group was significantly older suggesting that they had a more severe disease than their peer groups without, or with 1 pathogen found. It is obvious that not all possible pathogens found will cause illness, although it is still not clear whether and how the presence of a colonizing potential respiratory pathogen influences or facilitates other possible pathogens. It is therefore important to extend studies on respiratory infections with more than one pathogen to evaluate the influence on disease severity.

References

1. Jongste JC de, Shields MD. Cough. 2: Chronic cough in children. *Thorax* 2003; **581**: 998-1003.

2. Wirsing von König CH, Rott H, Bogaerts H, Schmitt HJ. A serological study of organisms possibly associated with pertussis-like coughing. *Pediatr Infect Dis J* 1998; **17**: 645-649.

3. Aoyama T, Ide Y, Watanabe J, Takeuchi Y, Imaizumi A. Respiratory failure caused by dual infection with *Bordetella pertussis* and respiratory syncytial virus. *Acta Paediat Japon* 1996; **38**: 282-285.

4. Moshal KL, Hodinka RL, McGowan KL. Concomitant viral and *Bordetella pertussis* infections in infants. *Pediatr Infect Dis J* 1998; **17**: 353-354.

5. Dagan R, Hall CB, Menegus MA. Atypical bacterial infections explained by a concomitant virus infection. *Pediatrics* 1985; **76**: 411-414.

6. Tristram DA, Miller RW, McMillan JA, Weiner LB. Simultaneous infection with respiratory syncytial virus and other respiratory pathogens. *Am J Dis Child* 1988; **142**: 834-836.

7. Thomson F, Masters IB, Chang AB. Persistent cough in children and the overuse of medications. *J Paediatr Child Health* 2002; **38**: 578-581.

8. Brémont F, Micheau P, Le Roux P, Brouard J, Pin I, Fayon M. Étiologie de la toux chronique de l'enfant: analyse de 100 dossiers. *Arch Pediatr* 2001; **8** (suppl 3): 645-649.

9. Hallander HO, Gnarpe J, Gnarpe H, Olin P. *Bordetella pertussis*, *Bordetella parapertussis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and persistent cough in children. *Scand J Infect Dis* 1999; **31**: 281-286.

10. Gottfarb P, Brauner A. Children with persistent cough--outcome with treatment and role of *Moraxella catarrhalis*? *Scand J Infect Dis* 1994; **26**: 545-551.

11. Gilberg S, Njamkepo E, Parent du Chatelet I, Partouche H, Gueirard P, Ghasarossian C, Schlumberger M, Guiso N. Evidence of *Bordetella pertussis* infection in adults presenting with persistent cough in a French area with very high whole-cell vaccine coverage. *J Infect Dis* 2002; **186**:415-418.

12. Strebel P, Nordin J, Edwards K, Hunt J, Besser J, Burns S, Amundson G, Baughman A, Wattigney W. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995-1996. *J Infect Dis* 2001; **183**:1353-1359.

13. Nagel J, Graaf S de, Schijf-Evers D. Improved serodiagnosis of whooping cough caused by *Bordetella pertussis* by determination of IgG anti-LPF antibody levels. *Dev Biol Stand* 1985; **61**: 325-330.

14. Melker HE de, Versteegh FGA, Conyn-Van Spaendonck MA, Elvers LH, Berbers GA, van der Zee A, Schellekens JFP. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. *J Clin Microbiol* 2000; **38**:800-806.

15. Herrmann KL. *Antibody detection*. In: Lennette EH, Halonen P, Murphy FA, eds. *Laboratory diagnosis of Infectious diseases. Principles and practice.* Vol. II. Berlin: Springer Verlag. 1988: 76-101.

16. Zee A van der, Agterberg C, Peeters M, Mooi F, Schellekens J. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. *J Infect Dis* 1996; **174**: 89-96.

17. Zee A van der, Agterberg C, Peeters M, Schellekens J, Mooi FR. Polymerase chain reaction assay for pertussis: simultaneous detection and discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. *J Clin Microbiol* 1993; **31**: 2134-2140.

18. Dorigo-Zetsma JW, Zaat SAJ, Wertheim-van Dillen PME, Spanjaard L, Rijntjes J, Waveren G van, Jensen JS, Angulo AF, Dankert J. Comparison of PCR, culture and serological tests for diagnosis of *Mycoplasma pneumoniae* respiratory tract infection in children. *J Clin Microbiol* 1999; **37**: 14-17.

19. Meijer A, Vliet JA van der, Schouls LM, Vries A de, Roholl PJ, Ossewaarde JM. Detection of microorganisms in vessel wall specimens of the abdominal aorta: development of a PCR assay in the absence of a gold standard. *Res Microbiol* 1998; **149**: 577-583.

20. Andeweg AC, Bestebroer TM, Huybreghs M, Kimman TG, Jong JC de. Improved detection of rhinoviruses in clinical samples by using a newly developed nested reverse transcription-PCR assay. *J Clin Microbiol* 1999; **37**: 524-530.

21. Fouchier RAM, Bestebroer TM, Herfst S, Kemp L van der, Rimmelzwaan GF, Osterhaus ADME. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. *J Clin Microbiol* 2000; **38**: 4096-4101.

22. Claas EC, Sprenger MJ, Kleter GE, Beek R van, Quint WG, Masurel N. Type-specific identification of influenza viruses A, B and C by the polymerase chain reaction. *J Virol Methods* 1992; **39**: 1-13.

23. Hoogen BG van den, Jong JC de, Groen J, Kuiken T, Groot R de, Fouchier RAM, Osterhaus ADME. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001; **7**: 719-724.

24. Cubie HA, Inglis JM, Leslie EE, Edmunds AT, Totapally B. Detection of respiratory syncytial virus in acute bronchiolitis in infants. *J Med Virol* 1992; **38**: 283-287.

25. Myint S, Johnston S, Sanderson G, Simpson H. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. *Mol Cell Probes* 1994; **8**: 357-364.

26. Bartlett JG, Ryan KJ, Smith TF, Wilson WR. Laboratory diagnosis of lower respiratory tract infections. Cumitech 7A: pp13-14. *American Society for Microbiology* September 1987.

27. Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of diseaseburden. *BMJ* 1997; **315**: 1060-1064.

28. Jartti T, Lehtinen P, Vuorinen T, Osterback R, Hoogen B van den, Osterhaus ADME, Ruuskanen O. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004; **10**: 1095-1101.

29. Benten I van, Koopman L, Niesters B, Hop W, van Middelkoop B, de Waal L, Drunen K van, Osterhaus A, Neijens H, Fokkens W. Predominance of rhinovirus in the nose of symptomatic and

asymptomatic infants. *Pediatr Allergy Immunol* 2003; **14**: 363-370.

30. Heijnen MLA, Dorigo-Zetsma JW, Bartelds AIM, Wilbrink B, Sprenger MJW. Surveillance of respiratory pathogens and influenza-like illnesses in general practices - The Netherlands, winter 1997/98. *Euro Surveill* 1999; **4**: 81-84.

31. Gunnarsson RK, Holm SE, Söderström M. The prevalence of potentially pathogenic bacteria in nasopharyngeal samples from individuals with a long-standing cough-clinical value of a nasopharyngeal sample. *Fam Pract* 2000; **17**: 150-155.

32. Azevedo AMN, Durigon EL, Okasima V, Queiroz DAO, Moraes-Vasconcelos D de, Duarte AJS, Grumach AS. Detection of influenza, parainfluenza, adenovirus and respiratory syncytial virus during asthma attacks in children older than 2 years old. *Allergol Immunopathol (Madr)* 2003; **31**: 311-317.

33. Nelson KE, Gavitt F, Batt MD, Kallick CA, Reddi KT, Levin S. The role of adenoviruses in the pertussis syndrome. *J Pediatr* 1975; **86**: 335-341.

34. Sturdy PM, Court SDM, Gardner PS. Viruses and whooping-cough. *Lancet* 1971; **2(7731)**: 978-979.

35. Connor JD. Evidence for an etiologic role of adenoviral infection in pertussis syndrome. *NEJM* 1970; **283**: 390-394.

36. Gageldonk- Lafeber AB van, Bartelds A, Heijnen MLH, Peeters MF, Plas SM van der, Greeff SC de, Wilbrink B. A case-control study on acute respiratory infections of patients in general practices in the Netherlands, October 2000-September 2003. *Clin Microbiol Inf* 2004; **10**(suppl 3): 91-92.

37. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH, Tyrrell DAJ, Holgate ST. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995; **310**: 1225-1229.

38. Thumerelle C, Deschildre A, Bouquillon C, Santos C, Sardet A, Scalbert M, Delbecque L, Debray P, Dewilde A, Turck D, Leclerc F. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: a prospective study in the Nord-Pas de Calais region (France). *Pediatr Pulmonol* 2003; **35**: 75-82.

39. Wesley AG, Windsor IM. Viral infections in clinical pertussis. *S Afr Med J* 1983; **64**: 577-578.

40. Banerji A, Bell A, Mills EL, McDonald J, Subbarao K, Stark G, Eynon N, Loo VG. Lower respiratory tract infections in Inuit infants on Baffin Island. *CMAJ* 2001; **164**: 1847-1850.

41. Keller MA, Aftandelians R, Connor JD. Etiology of pertussis syndrome. *Pediatrics* 1980; **66**: 50-55.

42. Cimolai N, Wensley D, Seear M, Thomas ET. *Mycoplasma pneumoniae* as a cofactor in severe respiratory infections. *Clin Infect Dis* 1995; **21**: 1182-1185.

43. Drews AL, Atmar RL, Glezen WP, Baxter BD, Piedra PA, Greenberg SB. Dual respiratory virus infections. *Clin Infect Dis* 1997; **25**: 1421-1429.

44. Juvén T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivanen M, Eskola J, Saikku P, Ruuskanen O. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 2000; **19**: 293-298.

45. Hakonarson H, Maskeri N, Carter C, Hodinka RL, Campbell D, Grunstein MM. Mechanism of rhinovirus-induced changes in airway smooth muscle responsiveness. *J Clin Invest* 1998; **102**: 1732-1741.

46. Tsolia MN, Psarras S, Bossios A, Audi H, Paldanius M, Gourgiotis D, Kallergi K, Kafetzis DA, Constantopoulos A, Papadopoulos NG: Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 2004; **39**: 681-686 Epub 2004 Aug 13

47. Hoek L van der, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC, Wertheim-Van Dillen PME, Kaandorp J, Spaargaren J, Berkhout B Identification of a new human corona virus. *Nat Med* 2004; **10**: 368-373.

48. Mejías A, Chávez-Bueno S, Ramilo O. Human metapneumovirus: a not so new virus. *Pediatr Infect Dis J* 2004; **23**: 1-7.

49. Bogaert D, Engelen MN, Timmers-Reker AJM, Elzenaar KP, Peerbooms PGH, Coutinho RA, Groot R de, Hermans PWM. Pneumococcal carriage in children in The Netherlands: a molecular epidemiological study. *J Clin Microbiol* 2001; **39**: 3316-3320.

Chapter 10

Summary and Conclusions

Summary and Conclusions

In this thesis we report the results of the studies we performed to answer the questions, which were put forward in chapter 1. The first part of this thesis focuses on the interpretation of *Bordetella (B.) pertussis* serology and the epidemiological consequences of our results evaluating surveillance data. The second part of this thesis shows data on other respiratory pathogens besides *B.pertussis* in mixed infections and prolonged coughing.

In this last chapter the most relevant findings will be summarized following the questions from chapter 1. Also practical implications and suggestions for future research will be proposed.

1: which titer of immunoglobulin G antibodies against pertussis toxin (IgG-PT) in one point serology is proof for recent infection?

Our results show that an IgG-PT level of at least 100 U/ml is a specific tool in laboratory confirmation by one-point serology of patients with a suspected pertussis infection. Independently of age, these levels are diagnostic of very recent or actual infection with *B.pertussis*. Such levels are present in less than 1% of the population and are reached in most pertussis patients within 4 weeks after disease onset. High IgG-PT levels persist only temporarily. The IgG-PT levels after natural infection with *B.pertussis* decrease within less than 1 year to a level below 100 U/ml for almost all patients who had high IgG-PT levels. The regression model we used in this study predicts that peak levels > 100 U/ml occur 4-8 weeks after infection. In the declining phase a level of 100 U/ml is reached in 4.5 months after infection onset. A level of <40 U/ml is reached within 1 year after disease onset. At diagnosis the number of patients with IgG-PT levels ≥ 100 U/ml is considerably larger (4.5-fold) than the number of patients with at least a 4-fold increase in IgG-PT. IgG-PT levels > 100 U/ml provide a useful laboratory tool for the diagnosis of pertussis in both the individual patient as in epidemiological studies.

2: may a patient suffer from *B.pertussis* infection more than once in a lifetime?

In chapter 3 we demonstrate that children, vaccinated or not, may have serologic evidence of re-infection with *B.pertussis*. Although it is known that people may suffer from re-infection with *B.pertussis*, these patients are the first in whom symptomatic re-infection with *B.pertussis* has definitely been proven. The infection took place 3.5-12 years after the first infection. But it is obvious that clinical complaints not necessarily comply with typical pertussis infection. It was shown that the severity and duration of respiratory symptoms in patients with proven and relatively early re-infections with *B.pertussis* increased with time elapsed since the first infection.

B.pertussis infection should be considered in patients with symptoms of typical or atypical

whooping cough, irrespective of age, their vaccination status or previous whooping cough.

3: what is the natural course of antibodies against *B.pertussis* after infection?

Evolution in data evaluation:

In the chapters 2, 4 and 5 in this thesis we have used different ways of evaluating the decline of immunoglobulin G to pertussis toxin (IgG-PT) after natural infection with *B.pertussis*. These 3 methods reflect gradual improvement and advancing understanding. In response to an infection, IgG titers typically show a rapid increase, followed by a steady, slow decline over several years. IgG-PT responses appear to show considerable variation among individual patients.

In chapter 2 we used a linear mixed model (PROC Mixed in SAS version 6.12) to analyze the association between ^{2}log IgG-PT levels and time in ^{2}log days after the first day of illness. The rapid increase of IgG-PT in the first weeks after illness was not taken in account, nor were effects of age or vaccination status. Fitting a straight line to the log-log transformed data can only account for the decaying phase of the response and requires the omission of data from the initial rapid increase. Since there is no clearly defined criterion by which observations can be excluded, the following study (chapter 4) continued with a response model, that includes the initial rising phase.

In chapter 4 we assumed that the slow decline in IgG antibody titers with time following infection disallows use of a first order model of antibody decay. On a log time – log titer scale such a model would lead to a convex curve, with an increasing downward slope. For our data, such a slope might be too steep, leading to a poor fit to the observed responses. The model we used, a skewed hyperbola, has asymptotically linear decline on a log-log scale at long times from infection. In this model there seems a significant effect of age or vaccination status but because the numbers of adults and unvaccinated infants were small and because there was a wide variation of data it remains unclear whether this is clinically relevant.

For any individual patient the amount of information is limited: only a few measurements at best. Although the previous regression model adequately fitted the data, this model did not help much in interpreting the observed immune responses. For that reason we continued with a biologically based model in chapter 5. We describe longitudinal responses with a dynamic model of the interaction between bacteria and the immune system. The model assumes that during infection bacteria are growing exponentially in the host. At the same time, bacteria are inactivated (killed) by antibodies (or by some action associated with the antibody response) according to a mass-action mechanism: inactivation depends on the product of the concentrations of bacteria and antibodies (or antibody producing cells). Conversely, antibody production is controlled by the probability of antibodies (or antibody producing cells) encountering bacteria (or being presented with antigens derived from these pathogens). Antibody removal is considered an autonomous first order process. This so-called predator-

prey model is the simplest possible model for the interaction between host and pathogen. Combining data from patients aged 0-94 years, we found no significant differences in rise, peak and decline of IgG-PT between different age groups. But there seems a tendency to age-related differences where older people tend to have a more rapid increase, a higher peak and a faster decline after infection than younger age groups. This could be caused by immunological memory.

4: what is the yearly incidence of *B.pertussis* infection?

In chapter 2 we already showed that an IgG-PT level of at least 100 U/ml is present in less than 1% of the population. In chapter 6, using the statistical model described in chapter 4 on the IgG-PT data of a cross-section of the general Dutch population we conclude that *B.pertussis* infections occur frequently in the Dutch population, particularly in adults for whom the reported incidence is very low. On average the estimated incidence of infection is 6.6% per year for 3 to 79-years olds, where as the annual incidence of notified cases was 0.01%! The age-distribution of all infections differs notably from the age-distribution of notified cases. We therefore suggest that vaccination strategies should not be based on notification data but on knowledge about the circulation of *B.pertussis* in different age groups and contact patterns between age groups.

5: what is the best way to protect newborns and not yet (fully) vaccinated babies against *B. pertussis* infection?

In chapter 7 we review the risks of perinatal infection with *B.pertussis*, the diagnosis, treatment and prophylaxis. Also we consider the possible influence of different vaccination schemes on the rate of *B.pertussis* infection in young children and the disease burden in older age groups. Although the best way seems to be to vaccinate the adolescent and adult population every 10 years, the feasibility of this regime requires large investments in making people aware of the necessity of these booster vaccinations and to develop a new infrastructure to deliver these vaccinations. Another option is to vaccinate pregnant women or women before pregnancy and their families.

6: how often mixed infections occur in *B.pertussis* infection?

During the prospective study on the longitudinal course of IgG-PT we regularly observed mixed infections. Therefore in chapter 8 we retrospectively looked for mixed infections in this group and in a group of patients in whom the diagnosis pertussis was made in 1998. In 28 % of the patients with proven *B.pertussis* infection there was evidence of mixed infection with para-influenza virus, RSV, *Mycoplasma pneumoniae*, adenovirus or influenza virus. Since infection with more than one pathogen might cause more severe or prolonged disease we suggest the possibility of dual infection should be investigated more often.

7: what is the role of other respiratory pathogens in the pertussis like syndrome?

To investigate the role of different respiratory pathogens in prolonged coughing in children and to analyze the clinical impact of mixed infections of *B.pertussis* with other respiratory pathogens we performed a prospective study in children with coughing complaints lasting 1 to 6 weeks (chapter 9). One third of the patients had one pathogen (n=49, 36%), one third more respiratory pathogens (n=42, 31%). The most frequent pathogens encountered were rhinovirus, *B.pertussis* and respiratory syncytial virus. In the patients with a mixed infection the most frequent combination was *B.pertussis* and rhinovirus. Infections with more than one pathogen occurred during the whole year regardless of the season. No signs of more disease severity could be demonstrated in children with more than 1 pathogen, although children with more than 1 pathogen were significantly older than those with none or 1 pathogen. There were no clinical data found that discriminated between pathogens, whether pathogens were found or not, or differences in treatment.

The threshold for IgG-PT of 100 U/ml as proof of recent infection made it possible to improve the diagnosis of *B.pertussis* infection, by one-point serology. It becomes clear from the long term data on notification of *B.pertussis* infection, two-point and one-point serology that the discrepancy between notifications and positive serology became smaller since the acceptation of one-point serology and the notification rate has increased. Before 1996 all serological tests for pertussis were performed at the LIS-RIVM. However, since 1998 at least three of the 16 regional Public Health Laboratories and also some other (hospital) laboratories have started to perform serology with commercial available assays. Consequently, the population coverage of serological surveillance based on serological data of LIS-RIVM is now estimated to have decreased from 100% to less than 50%. This explains why the notification rate since 1999 is higher than the number of patients with positive serology at LIS-RIVM. Because there is no longer one central laboratory for the diagnosis of *B.pertussis* infections it is more difficult to gain insight in the epidemiology of *B.pertussis* infections in the Netherlands; notification data differ significantly from estimated infections and are very much lower.

It became clear that people may suffer from *B.pertussis* infection more than once in a lifetime. When the time between infections is longer the more severe disease occurs in the second infection. There is some evidence that the moment of re-infections might be influenced by booster vaccination against *B.pertussis*. This, together with the increasing awareness about the importance of adolescents and adults as a source of *B.pertussis* infection, makes it important to gain more insight in the incidence of *B.pertussis* infection and the influence of booster vaccination. Therefore studies should be designed to investigate this interaction in order to develop new vaccination strategies.

The development and validation of models of the kinetics of IgG-PT after *B.pertussis* infection makes it possible to compare patients of different ages, from different places or different

time periods. This will help to increase the knowledge about the epidemiology of *B.pertussis* infection. Since there seems a tendency of an age dependent course of IgG-PT after *B.pertussis* infection further studies are needed to confirm this finding. If this age dependency holds true it influences the results of demographic studies about the incidence of *B.pertussis* infection. It might also influence decisions about vaccination schedules for adult booster vaccination. Comparison of the different methods used in this thesis to calculate the course of IgG-PT after *B.pertussis* infection is therefore the first step. When this swifter and higher response in older people means they will suffer from *B.pertussis* infection with less pronounced disease and therefore will not as easily be recognized as pertussis patients, more attention should be paid to identify *B.pertussis* infection in this age group.

Although there is a significant number of mixed infections both in *B.pertussis* infection and in prolonged coughing, we could not demonstrate more severe disease in mixed infection. Also many people are carrier of respiratory infectious agents without disease. This leaves open the question of the relevance of mixed infections. When mixed infections are not true mixed infections but consecutive infections they still may prolong disease. Also an increased knowledge about mixed infection might influence the management of patients.

Most important is how to control *B.pertussis* infection, especially for the vulnerable, not or partially vaccinated, young children. Isolating patients is effective but impossible to execute regarding the number of patients and the difficulty to identify the majority of the older patients because of their aspecific or subclinical symptoms. Another possibility is antibiotic prophylaxis for the ones at risk and all people around this patient, or only for those at risk. Although passive immunization with human hyperimmune anti-pertussis gammaglobulin is still used occasionally elsewhere, it is no common practice in the Netherlands, where the hyperimmune anti-pertussis gammaglobulin is not available.

The best way to control *B.pertussis* infection is vaccination either with whole-cell or acellular vaccine. Since asymptomatic re-infection may occur rather a short period after infection or vaccination protective immunity against disease lasts longer than the immunity against infection. Thus it seems that natural infection or vaccination provides better protection against the toxic effects of *B.pertussis* infection than against adhesion but the protection is not life-long. The duration of the protective immunity acquired by vaccination lasts about as long as by natural infection (4-12 years vs. 4-20 years), although data are not unequivocal.

The interaction between vaccination and boosting of pertussis immunity by *B. pertussis* infection on one hand and waning immunity against *B. pertussis* on the other influences the epidemiology and the dynamics of pertussis in the population. Therefore it is necessary to study the influences of changes in vaccination schedules.

The studies presented in this thesis made us understand better some things about *B.pertussis* infection but also raised new questions. New studies have to be performed to answer some of these questions but for sure will raise other ones.

Kinkhoest: nieuwe inzichten in diagnose, voorkomen en klinische aspecten

Samenvatting

Inleiding

Kinkhoest is een besmettelijke ziekte van de luchtwegen, veroorzaakt door de kinkhoest bacterie: *Bordetella pertussis*. Deze infectie kan voorkomen op alle leeftijden, maar de klachten zijn het ergst bij kleine kinderen. Jaarlijks hebben wereldwijd 20-40 miljoen mensen kinkhoest, vooral in de ontwikkelingslanden. Er wordt geschat dat per jaar ongeveer 200 000-400 000 mensen aan kinkhoest overlijden.

De eerste epidemie van kinkhoest is beschreven door Guillaume de Baillou (1538-1616) in 1578, maar al veel eerder, in 1190, was in Engeland een ziektebeeld bekend dat kinkehost werd genoemd. En Dodonaeus (1517-1585) beschrijft in zijn Cruijde boeck uit 1554 al een therapie voor kieckhoest!

Kinkhoest manifesteert zich als een heftig hoesten met stikbuien, maar afhankelijk van voorafgaande vaccinaties en/of eerdere infecties kan de ziekte milder verlopen. Het klinische beeld kent drie stadia, 1: de catarrale fase, gekenmerkt door een verkoudheidsbeeld en beginnend hoesten, 2: de paroxysmale fase, met de typische hoestaanvallen, en 3: de herstelfase, waarin de klachten langzaam afnemen. De totale ziekteduur bedraagt ongeveer drie maanden. In het Chinees wordt de ziekte dan ook de "honderd dagen ziekte" genoemd.

De bacteriesoort Bordetella kent op dit moment acht verschillende typen: *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Bordetella avium*, *Bordetella hinzii*, *Bordetella holmesii*, *Bordetella trematum* en *Bordetella petrii*. *Bordetella pertussis* komt alleen bij de mens voor, de andere komen vooral bij dieren voor maar kunnen ook klachten geven bij mensen, vooral de *Bordetella parapertussis*.

Bij een infectie met *Bordetella pertussis* komt een aantal ziekmakende stoffen vrij, zoals onder andere pertussis toxine, filamenteus haemagglutinine, fimbriae en pertactin. Tegen deze stoffen, antigenen, vormt het lichaam antistoffen die een rol spelen in de immuniteit tegen infectie met *Bordetella pertussis*. Het aantonen van deze antistoffen wordt gebruikt in de diagnostiek van kinkhoest.

De afgelopen tien jaar is er een duidelijke toename van het aantal kinkhoestgevallen geconstateerd, vooral in ontwikkelde landen met een hoge vaccinatiegraad. In Nederland was tussen 1989 en 1994 de gemiddelde incidentie (aantal nieuwe gevallen) van kinkhoest 2,4 per 100.000 mensen op basis van meldingen en 2,3 per 100.000 mensen op basis van positieve serologie (aantonen van antistoffen in bloed). In 1996 was er sprake van een sterke stijging van de incidentie tot 27.3 per 100.000 en sindsdien is deze hoog gebleven. Een belangrijke oorzaak voor de toename van *Bordetella pertussis* infecties is dat er veranderingen zijn opgetreden in

het genoom (complex van erfelijke factoren) van de *Bordetella pertussis* na het starten van de vaccinaties in de vijftiger jaren.

Kinkhoest is voortdurend aanwezig in de bevolking, en daarbovenop komen epidemieën voor met een cyclus van ongeveer 4 jaar in landen waar gevaccineerd wordt en een cyclus van 2 tot 3 jaar elders. In Nederland zijn deze cycli, ondanks de vaccinatie, sinds 1996 ook om de 2 à 3 jaar. *Bordetella pertussis* is erg besmettelijk en wordt vooral door hoestdruppeltjes van mens op mens overgedragen.

Natuurlijke infectie of vaccineren geeft een langdurige maar geen levenslange bescherming tegen kinkhoestinfectie. Door gedeeltelijke bescherming kan een milder ziektebeeld ontstaan, dat vaak niet als kinkhoest herkend wordt. Dit is vooral het geval bij adolescenten en volwassenen. Deze mensen kunnen echter wel anderen besmetten, vooral ongevaccineerde of gedeeltelijk gevaccineerde jonge kinderen.

De diagnose kinkhoest wordt gesteld op klinische symptomen: langer dan twee weken hoesten, met daarbij of hoestbuien, en/of kinken (gierende inademing) en/of overgeven direct na het hoesten. De diagnose kinkhoest kan bevestigd worden door het kweken van de *Bordetella pertussis* of door het aantonen van deze bacterie middels de polymerase chain reaction (PCR). Ook kunnen er antistoffen tegen antigenen in het bloed aangetoond worden. Daarbij moet in twee verschillende bloedmonsters minstens een viervoudige stijging van de antistoffen optreden. Een positieve kweek met *Bordetella pertussis* is de gouden standaard voor het vaststellen van kinkhoest, maar het kweken van de *Bordetella pertussis* is erg moeilijk. PCR maakt het mogelijk ook nog *Bordetella pertussis* aan te tonen als de bacterie niet meer leeft; maar 3 à 4 weken na de infectie is de kans op een positief resultaat van de kweek of PCR niet erg groot meer. Als de ziekte langer aanhoudt heeft het meer zin antistoffen in het bloed aan te tonen. Bepaling van de antistof immunoglobuline G tegen pertussis toxine (IgG-PT) is de meest gebruikte en best gevalideerde test. IgG-PT wordt uitsluitend aangemaakt na een infectie met *Bordetella pertussis*, niet met andere Bordetella's. Antistoffen tegen andere antigenen worden ook wel gebruikt, maar zijn minder gestandaardiseerd. Bovendien zijn deze antigenen ook bij infecties met andere Bordetella's te vinden. Het vaststellen van een viervoudige stijging van de antistoffen in het bloed wordt bemoeilijkt doordat veel patiënten pas later tijdens hun ziekte medische hulp zoeken. Dan hebben de antistoffen vaak al (bijna) hun piek bereikt (meestal na 4 à 5 weken) en kan geen viervoudige stijging meer worden aangetoond. Heel jonge kinderen hebben nog een onrijpe afweer. Daardoor wordt de bacterie langzamer uit het lichaam verwijderd en heeft het zin ook na langere tijd nog een kweek en/of PCR in te zetten.

Een klinisch beeld lijkend op kinkhoest kan ook veroorzaakt worden door andere verwekkers als adenovirus, influenzavirus, parainfluenza virus, respiratoir syncytiaal virus, *Chlamydia*

pneumoniae of *Mycoplasma pneumoniae*. Ook zijn gemengde infecties beschreven met verschillende verwekkers.

Kinkhoest kan behandeld worden met antibiotica, macroliden hebben daarbij de voorkeur. Vaccinatie beschermt tegen infectie met *Bordetella pertussis*. Meestal gebeurt dit met een combinatievaccin, waarin ook difterie, tetanus, en polio zitten. Jarenlang is gebruik gemaakt van vaccins op basis van geïnactiveerde bacteriën, het whole-cell vaccin. Vanwege de bijwerkingen zijn nieuwe vaccins ontwikkeld met daarin een of meer geselecteerde antigenen, zoals pertussis toxine. Dit zogenaamde acellulaire vaccin is in 2001 opgenomen in het Rijks Vaccinatie Programma.

Doel van dit proefschrift

Door de plotselinge stijging in 1996 van het aantal kinkhoest gevallen in Nederland stelden wij ons een aantal vragen:
- Kunnen we met één enkel bloedmonster de diagnose kinkhoest stellen?
- Kun je meer dan één keer in je leven kinkhoest krijgen?
- Hoe snel verdwijnen de antistoffen tegen kinkhoest na een infectie uit je bloed?
- Hoe vaak komen kinkhoest infecties elk jaar voor en bij wie?
- Hoe kun je de meest kwetsbaren (pasgeborenen en jonge kinderen die nog niet volledig zijn gevaccineerd) het best beschermen tegen kinkhoest?
- Hoe vaak komen menginfecties voor bij kinkhoest en zo ja welke verwekkers spelen een rol?
- Is ernstig hoesten altijd kinkhoest en wat is de rol van andere verwekkers van hoestklachten in het beeld lijkend op kinkhoest?

Opzet

Vanaf 1993 werd bij ongeveer 100 patiënten die kinkhoest hadden doorgemaakt, als er om een andere reden toch bloed afgenomen moest worden, na verkregen toestemming, wat extra bloed afgenomen voor het opnieuw bepalen van het IgG-PT. Daarnaast werd bij een deel van deze patiënten gekeken of er nog andere verwekkers van luchtweginfecties aangetoond konden worden. Deze gegevens werden op zich en in samenhang met andere gegevens bewerkt.
Daarnaast werd in een tweejarige prospectieve studie vanaf 2001 onderzocht welke verwekkers aangetoond konden worden bij kinderen met langdurig hoesten.

Resultaten

In *hoofdstuk 1* wordt een overzicht gegeven van de huidige stand van zaken met betrekking tot *Bordetella pertussis* infecties. Daarnaast wordt uiteen gezet wat de vraagstellingen zijn van dit proefschrift.

Hoofdstuk 2 beschrijft de studie waarin wij aantonen dat een titer (gehalte) van IgG-PT van 100 U/ml of hoger bewijzend is voor een recente *Bordetella pertussis* infectie, en dat daarmee voor de diagnose kinkhoest bepaling in één bloedmonster (eenpuntsserologie) mogelijk is. Minder dan 1% van de bevolking heeft titers boven de 100 U/ml. In het wiskundig model dat we gebruiken in deze studie bereikt de IgG-PT titer een piek na 4-8 weken, waarna een geleidelijke daling optreedt. Na ongeveer 4½ maand wordt in de dalende fase gemiddeld de 100 U/ml grens weer bereikt, en na een jaar bedraagt de gemiddelde titer < 40 U/ml. Het aantal patiënten met een IgG-PT titer > 100 U/ml in één bloedmonster is 4,5 maal zo groot als het aantal patiënten met een viervoudige titerstijging in twee bloedmonsters. Een IgG-PT titer > 100 U/ml is daarmee een zeer bruikbaar hulpmiddel in de diagnostiek van kinkhoest in de individuele patiënt èn in epidemiologische studies.

In *hoofdstuk 3* beschrijven we vier patiënten die serologisch bewezen voor de tweede keer een kinkhoest infectie doormaken, 3,5 tot 12 jaar na de eerste infectie. Drie van de vier kinderen zijn in hun eerste levensjaar gevaccineerd. Ofschoon wel vermoed werd dat mensen opnieuw kinkhoest kunnen doormaken, zijn deze patiënten de eersten waar dit ook in beide gevallen is bewezen. Bij deze patiënten was de tweede infectie niet altijd herkenbaar als een typische kinkhoestinfectie. Wel waren de klachten ernstiger en leken meer op een typische kinkhoestinfectie naarmate de eerste infectie langer geleden had plaatsgevonden. Het is dus belangrijk bij mensen met hoestklachten ook aan een kinkhoestinfectie te denken, ondanks eerdere vaccinatie en/of kinkhoestinfectie.

In de *hoofdstukken 2,4 en 5* zijn verschillende methoden/wiskundige modellen gebruikt om de daling van het IgG-PT te beschrijven. Deze drie methoden zijn het gevolg van opeenvolgende verbeteringen en voortschrijdend inzicht. In de in *hoofdstuk 2* gebruikte methode wordt uitsluitend rekening gehouden met de gegevens uit de dalende fase van het IgG-PT.

Daarom werd in *hoofdstuk 4* een model ontwikkeld dat ook gebruik maakt van de eerste stijgende fase van de antistofrespons. Het hierbij gebruikte model van een scheve hyperbool past beter op de gevonden data dan het eerste model, maar geeft nog steeds weinig aanknopingspunten bij het interpreteren van de gevonden immuun respons.

Vervolgens werd in *hoofdstuk 5* het beloop van de antistofrespons in de tijd beschreven middels een dynamisch model van de interactie tussen de bacterie en het immuunsysteem. Dit model gaat er van uit dat de bacterie exponentieel groeit in de gastheer. Tegelijkertijd echter wordt de bacterie onschadelijk gemaakt door de antistofreactie. Het onschadelijk

maken van de bacterie hangt af van de kans dat antistoffen bacteriën tegenkomen. Daarnaast hangt de productie van antistoffen af van de ontmoetingskans tussen antistoffen en bacteriën. Dit zogenaamde roofdier-prooimodel is het meest eenvoudige model om de interactie tussen gastheer en ziekteverwekker te beschrijven.

In *hoofdstuk 5* wordt het beloop van IgG-PT in een groep kinkhoestpatiënten van 0 tot 94 jaar beschreven. Hierbij werden geen significante verschillen gevonden tussen de verschillende leeftijdsgroepen wat betreft stijging, piek en daling van het IgG-PT. Maar er was wel een tendens dat oudere mensen een snellere stijging, een hogere piek en een snellere daling van het IgG-PT lieten zien. Dit zou kunnen wijzen op een vorm van immunologisch geheugen. Een goed wiskundig model is onder andere belangrijk voor het stellen van de diagnose maar ook bij het ontwikkelen van vaccinatiestrategieën.

Een belangrijke vraag is hoe vaak komt kinkhoest nu eigenlijk voor?

Gebruik makend van de IgG-PT data van een doorsnede van de Nederlandse bevolking, zoals beschreven in *hoofdstuk 2*, en met behulp van het model uit *hoofdstuk 4*, wordt in *hoofdstuk 6* berekend dat jaarlijks 6,6% van alle 3 tot 79 jarigen in Nederland kinkhoest doormaken. Echter, jaarlijks worden maar 0,1% kinkhoestinfecties gerapporteerd! Ook is er een groot verschil in leeftijdsverdeling tussen de berekende en gerapporteerde kinkhoestinfecties. De meeste gerapporteerde gevallen zijn jonger dan 1 jaar en tussen het 3 en 4e jaar, volgens de modelberekening ligt het hoogste aantal infecties in de leeftijd van 20 tot 24 jaar. De meeste overdracht vindt plaats bij jonge volwassenen: daarbij gaat het om infecties met weinig of geen symptomen, die daarom ontsnappen aan herkenning en rapportage. Het is dus belangrijk dat beschermingsstrategieën niet worden gebaseerd op het aantal gemelde kinkhoestpatiënten, maar op kennis van en inzicht in het voorkomen van *Bordetella pertussis* in de verschillende leeftijdsgroepen en hoe de contacten tussen de verschillende leeftijdsgroepen plaatsvinden.

In *hoofdstuk 7* wordt een overzicht gegeven van de risico's van kinkhoestinfecties in de zwangerschap en rondom de bevalling, de diagnostiek, behandeling en profylaxe. Tenslotte worden een aantal vaccinatiestrategieën besproken om met name de circulatie van *Bordetella pertussis* in de bevolking te reduceren en daarmee de risico's op een kinkhoestinfectie voor de risicogroepen te verminderen.

Gedurende de studie over het beloop in de tijd van de IgG-PT na een kinkhoestinfectie zagen we regelmatig een gemengde infectie. In *hoofdstuk 8* beschrijven we retrospectief het voorkomen van gemengde infecties in deze groep patiënten en een groep kinkhoestpatiënten uit 1998. Hierbij werd in 28% van de patiënten met een bewezen kinkhoestinfectie tevens een infectie gevonden met parainfluenza virus, respiratoir syncytiaal virus, *Mycoplasma pneumoniae*, adenovirus of influenza virus. Omdat gemengde infecties ernstigere of langere ziekte zouden kunnen veroorzaken is het belangrijk meer aandacht te besteden aan gemengde infecties.

Om de rol van verschillende verwekkers van luchtweginfecties te onderzoeken bij kinderen

met langdurig hoesten en om te zien wat de klinische invloed is van gemengde infecties van *Bordetella pertussis* met andere verwekkers van luchtweginfecties werd een tweejarige prospectieve studie opgezet.

Deze studie wordt in *hoofdstuk 9* beschreven. Bij ongeveer een derde van de patiënten werd geen verwekker (n=45, 33%) aangetoond, bij een derde één verwekker (n=49, 36%), en bij een derde meerdere (n=42, 31%). De meest voorkomende verwekkers zijn rhinovirus, *Bordetella pertussis* en respiratoir syncytiaal virus. Bij patiënten met een gemengde infectie is de meest voorkomende combinatie *Bordetella pertussis* en rhinovirus. Gemengde infecties komen het hele jaar voor ongeacht het seizoen. Kinderen met meer dan een verwekker zijn niet zieker, al zijn ze wel significant ouder dan kinderen zonder aantoonbare verwekker of slechts één verwekker. Ook zijn er geen klinische gegevens gevonden die een onderscheid mogelijk maken tussen de verschillende verwekkers, de aanwezigheid van verwekkers, of verschillen in behandeling.

Het feit dat een IgG-PT titer van 100 U/ml in een enkel bloedmonster een bewijs is van een recente kinkhoestinfectie heeft de diagnostiek van kinkhoest verbeterd en vereenvoudigd. Ook konden we aantonen dat mensen vaker dan één keer kinkhoest kunnen krijgen, gevaccineerd of niet. Tevens bleek dat hoe langer de tijd was tussen twee kinkhoestinfecties hoe zieker de patiënt de tweede keer was. Het modelleren van het beloop van IgG-PT titers na een kinkhoestinfectie maakt het mogelijk patiënten van verschillende leeftijden, verschillende plaatsen en verschillende tijdvakken te vergelijken. Hierdoor zal onze kennis over de epidemiologie van kinkhoest verder toenemen. Wel zal er verder onderzoek gedaan moeten worden naar het mogelijke leeftijdsbepaalde beloop van IgG-PT. Het door ons gevonden leeftijdspatroon heeft invloed op de uitkomsten van studies naar het voorkomen van kinkhoest en op de vaccinatieschema's voor volwassenen. De snellere en hogere reactie bij ouderen op een kinkhoestinfectie betekent dat zij minder ernstig ziek worden en daardoor ook moeilijker te herkennen zijn als een kinkhoestpatiënt. Er moet daarom extra aandacht besteed worden om kinkhoest in deze leeftijdsgroep te kunnen herkennen. Zowel bij kinkhoestinfecties als bij langdurig hoesten bestaan een groot aantal gemengde infecties. Er zijn echter geen aanwijzingen voor ernstigere ziekte bij gemengde infecties. Ook zijn veel mensen drager van een verwekker van luchtweginfecties zonder dat zij ziek zijn. Is er dan wel sprake van gemengde infecties? Meer kennis omtrent gemengde luchtweginfecties kan van invloed zijn op de behandeling van de patiënt.

Het belangrijkste is kinkhoest onder controle te krijgen ter bescherming van het jonge, niet of gedeeltelijk gevaccineerde, kind. De beste manier hiervoor is vaccinatie. Aangezien een klachtenvrije herinfectie met *Bordetella pertussis* al kort na een infectie of vaccinatie kan ontstaan lijkt de bescherming tegen ziek worden langer te bestaan dan de bescherming tegen infectie. Maar deze bescherming is niet levenslang. De interactie tussen vaccinatie en het

versterken van de immuniteit door natuurlijke infectie met *Bordetella pertussis* aan de ene kant en de verdwijnende immuniteit na verloop van tijd aan de andere kant is van invloed op de epidemiologie en de dynamiek van kinkhoest in de bevolking. Het is daarom belangrijk de door ons verzamelde gegevens te gebruiken voor het bestuderen van de invloed van veranderingen in vaccinatieschema's op de epidemiologie van kinkhoest.

Dankwoord

Woorden schieten tekort om iedereen te bedanken die meegeholpen heeft de verschillende onderzoeken goed te kunnen doen, maar toch ga ik een poging wagen.

Toch nog gepromoveerd. Eindelijk maakt het drs. (voor niet latinisten "hij die (nog) doctor moet worden") plaats voor Dr. Een mijlpaal waar ik niet meer op gerekend had. Dit proefschrift kon slechts een succes worden dankzij de hulp van heel veel mensen en instituten die ik enorm wil bedanken voor hun hulp. Een aantal wil ik in het bijzonder noemen, zonder anderen te kort te willen doen.

In de eerste plaats wil ik alle kinderen en ouders die meegedaan hebben aan de verschillende onderzoeken, bedanken voor hun bereidheid mee te doen en daarvoor soms vervelende onderzoeken te moeten ondergaan.

Prof.dr. J.J. Roord, beste John, op cruciale momenten in mijn carrière heb je een grote rol gespeeld. In 1989 gaf je mij het vertrouwen door me op te nemen in het team van de Algemene Pediatrie van het Wilhelmina Kinderziekenhuis, in 1999 gaf je me opnieuw het vertrouwen door je bereidheid uit te spreken mijn promotor te worden. In de vele jaren die je mij begeleid hebt op het pad naar dit proefschrift is het voortdurende vertrouwen dat je uitsprak dat het wel goed zou komen, een enorme steun geweest. Je "kwaliteit gaat voor snelheid" zal een leidraad blijven in mijn verdere carrière. Heel veel dank voor alles.

Dr. J.F.P. Schellekens, beste Joop, met enorm enthousiasme en tomeloze energie heb jij het afgelopen decennium geholpen bij het verzamelen van gegevens, het concipiëren van ideeën en het telkens blijven zoeken naar de best mogelijke oplossing en conclusie. Elk overleg was telkens weer een nieuw college kinkhoestkunde waar ik veel van geleerd heb. Je voortdurende steun heeft me geholpen ook de moeilijke momenten te overwinnen. Ik hoop dat dit proefschrift niet het einde is van onze samenwerking op kinkhoestterrein.

Dr. P.F.M. Teunis, beste Peter, heel veel dank voor je geduld mij als medicus-practicus te begeleiden op het moeilijke terrein van de biostatistiek. Hoe vaak moest iets niet over of anders en telkens lukte dat weer, ondanks je drukke andere werkzaamheden. We hebben nog wat losse eindjes liggen, ik kijk er naar uit die, samen met jou, ook nog aan elkaar te kunnen knopen.

Mw.dr. M.A. Mooijaart, Prof.dr. M.J. van Lieburg en Prof.dr. E. Houwaart wil ik hartelijk bedanken voor hun hulp bij het verzamelen van historische data.

Mijn collegae kinderartsen, J.C.M. Hoekx, Dr. E.H.G. van Leer, Dr. J.S. Starreveld, C.V. Tjon Pian Gi, beste Jurriaan, beste Cecil, de eerste jaren van onze samenwerking waren niet de makkelijkste. Ondanks onze aanvankelijke meningsverschillen hebben we altijd schouder aan schouder gestaan om de Kindergeneeskunde in Gouda op een hoog niveau te brengen en te houden en ik denk dat wij daarin behoorlijk geslaagd zijn. Heel veel dank voor heel veel collegialiteit en vriendschap.
Beste Ed, beste Sander, hoewel jullie pas korter deel uitmaken van onze vakgroep, is ook jullie steun onontbeerlijk geweest. Zeker Sanders hulp bij het opzoeken van literatuur heb ik erg op prijs gesteld.
Ik ben blij dat ik in dit team mag werken en hoop dat de goede sfeer die onze samenwerking al zo lang kenmerkt, ook de komende jaren de basis zal zijn voor nog heel veel mooie ontwikkelingen in onze praktijk.

Prof.dr. H.K.A. Visser, beste Henk, hartelijk wil ik je danken voor de opleiding die ik van je mocht genieten, waarmee de basis is gelegd die geleid heeft tot dit proefschrift.

Het secretariaat van de afdeling Kindergeneeskunde, de verpleging van de Kinderafdeling, alle arts-assistenten die de afgelopen jaren deel hebben uitgemaakt van ons team, alle medewerkers van het Klinisch Chemisch Laboratorium en alle medewerkers van Medisch-microbiologisch Laboratorium wil ik bedanken voor hun hulp bij het verzamelen en verwerken van de gegevens en voor de vele jaren van goede samenwerking.

Mijn lieve schoonouders, Ik wil jullie bedanken voor jullie dochter, jullie interesse en steun.

Lieve ouders, heel veel dank voor alles wat jullie in mij geïnvesteerd hebben. Deze mijlpaal is ook voor jullie.

Tot slot, lieve Machelien, jij weet dat ik weet dat jij weet wat jij voor mij betekent, en daar heeft verder niemand iets mee te maken. Dank voor je steun, jij had er soms meer vertrouwen in dat het zou lukken dan ik. En nu ga ik echt mijn kamer opruimen.

Lieve Frédérique, Jan en Hendt, de computer zal vanaf nu vaker vrij zijn. Dank voor jullie geduld en hulp, ik ben trots op jullie.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 13 oktober 1951 in Utrecht.

Na het behalen van het eindexamen Gymnasium ß op de R.K.Scholengemeenschap "de Breul" in Zeist in 1971, ging hij geneeskunde studeren aan de Rijksuniversiteit Groningen. Tijdens zijn studie was hij onder andere bestuurslid van de Groninger Studenten Roeivereniging "Aegir".

In 1979 haalde hij zijn doctoraal examen. Vervolgens vervulde hij zijn co-assistentschappen in het St. Elisabeth Hospitaal in Willemstad, Curaçao. Al vroeg bleek zijn voorliefde voor de kindergeneeskunde: hij deed, aansluitend aan zijn co-schappen, keuzestages neonatologie in het Rainbow Babies & Childrens Hospital, Case Western Reserve University in Cleveland (Prof. dr. A.A. Fanaroff, Prof. dr. R.J. Martin, Dr. W.A. Carlo) en kindercardiologie in het Childrens Hospital Medical Centre, Harvard Medical School in Boston (Prof. dr. A.S. Nadas, Dr. T.S. Hougen). In maart 1981 behaalde hij het artsexamen. Na terugkeer in Nederland werd hij van 1981 tot 1985 opgeleid tot kinderarts onder leiding van Prof. dr. H.K.A. Visser in het Sophia Kinderziekenhuis in Rotterdam.

Na zijn opleiding werkte hij tussen 1985 en 1991 in verschillende ziekenhuizen, onder andere het Academisch Medisch Centrum in Amsterdam en het Wilhelmina Kinderziekenhuis in Utrecht.

In 1991 ging hij werken in het St. Jozef Ziekenhuis in Gouda, dat na een fusie met het Bleuland Ziekenhuis opging in het Groene Hart Ziekenhuis. Daar werd vanaf 1993 in samenwerking met Dr. J.F.P. Schellekens (RIVM) en Prof. dr. J.J. Roord (VUmc) gewerkt aan de onderzoeken die geleid hebben tot dit proefschrift.

In de afgelopen jaren was hij in het ziekenhuis betrokken bij het opzetten van het Kinder Diabetes Team en de Transmurale AStmaverpleegkundigen voor Kinderen (TASK). Ook nam hij deel aan de totstandkoming van het Kinderastma Netwerk Midden Holland, en het Down Team Midden Holland. Daarnaast is hij sinds 1998 lid van de Medisch Ethische Commissie, vanaf 2002 als voorzitter.

De auteur is getrouwd met Machelien Bazuin. Zij hebben drie kinderen, Frédérique, Jan en Hendt.