Epidemiology of pertussis in the Netherlands and implications for future vaccination strategies

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Epidemiology of pertussis
in the Netherlands
and implications for future
vaccination strategies
Epidemiology of pertussis in the Netherlands and implications for future vaccination strategies

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof.dr. D.C. van den Boom

ten overstaan van een door het college voor promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op vrijdag 24 september 2010, te 10:00 uur
door

Sabine Christine de Greeff

goingen te Nijmegen
Promotiecommissie

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            Prof. dr. F.R. Mooi

Co-promotor: Dr. H.E. de Melker

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               Prof. dr. H.S.A. Heymans
               Prof. dr. T.W. Kuijpers
               Dr. L. Spanjaard
               Prof. dr. E.J. Ruitenber
               Prof. dr. E.A.M. Sanders

Faculteit der Geneeskunde
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Praying to Allowin van Haspengouw, also known as Saint Bavo, was thought to protect against pertussis. On the therapeutic value of that use a rhyme is written:

'Wie van de kinkhoest wil genezen,
ga naar Rijssbergen als voor dezen.
Sint Bavo wordt daarvoor geprezen.'

© Herzog Anton Ulrich-Museum, Braunschweig, Germany
History of pertussis epidemiology and vaccination

Pertussis is a highly contagious infectious disease of the respiratory tract. Typical pertussis illness usually starts after an incubation period of 7-20 days. The initial (catarrhal) phase is indistinguishable from common upper respiratory infections with two weeks of nasal congestion, rhinorrhea, and sneezing. In the following (paroxysmal) phase, with cough mostly lasting for more than 28 days, typical pertussis symptoms occur such as paroxysmal coughing, post-tussive vomiting and/or inspiratory whooping. In unvaccinated infants the infection is most severe and is often accompanied by cyanosis, apnoea, and seizures. In some cases severe complications may ensue such as pneumonia, encephalopathy or even death [1]. Although typical pertussis may occur, most older children and adults with pertussis report relatively mild disease with long term coughing and cold like symptoms [2, 3].

The first written description of a pertussis epidemic occurred in Paris and dates from 1578 [4]. In the subsequent centuries more epidemics in Europe were described, suggesting the disease expanded [5]. Anecdotic case reports and names of diseases that resemble the currently known names for whooping cough (kinkhoest, keuchhusten, coqueluche, pertussis, tussis quinta, tosse canina, tosse asinina) have been found in old dictionaries, articles, and medical books between the 16th and the 20th century [6-8]. At the time it was not clear what caused the disease and by some its contagiousness was ascribed to the involuntary imitation also observed with yawning: if one persons coughs, this may cause another person to “sympathetically” cough [9]. Later, in the 20th century it became clear that pertussis was caused by an infectious agent. The causative organism *Bordetella pertussis* was first isolated in 1906 by Bordet and Gengou and initially named *Haemophilus pertussis* [10]. Later, two other *Bordetella* species isolated from patients suspected of pertussis were identified, designated *Bordetella parapertussis* and *Bordetella bronchiseptica* [11, 12]. *B. bronchiseptica* is rarely isolated from pertussis patients and most of these infections are of zoonotic origin. However, like *B. pertussis*, *B. parapertussis* is a human pathogen. It generally causes a milder form of pertussis and in the Netherlands it is isolated from circa 5% of the pertussis patients.

Routinely collected data on disease frequency and distribution of pertussis are available since the 20th century. Before the introduction of vaccination, pertussis was a major cause of infant death worldwide. In 1934-1943, on average 4,000 deaths due to pertussis were reported each year in the US (3.2/100,000) and 350 in the Netherlands (3.5/100,000) [13, 14]. In both countries more than 95% of deaths occurred in children below 5 years of age. Although pertussis was mainly seen as a disease in childhood, clinical case data from the prevaccination era indicate that reinfections in adults were common [6]. In the 1940s, effective whole-cell vaccines against *B. pertussis* were developed, which were based on killed and detoxified bacteria. In the Netherlands, a single pertussis vaccine was produced by the National Institute...
for Public Health (RIV) in 1949, and from 1952 a combination vaccine against diphtheria, pertussis and tetanus became available (DTP) [13, 15]. In the beginning of the 1950s, the annual number of reported deaths due to pertussis decreased to thirty and cases of typical pertussis in children diminished, though atypical pertussis in immunized infants was still reported [16]. In 1953, DTP vaccination was free available for mass vaccination by general practitioners, child-care centres and school doctors, and the uptake of the vaccine increased in the beginning of the 1950s [13]. In 1955, an injectable polio vaccine (IPV) was registered in the US. After the epidemic of poliomyelitis in 1956 the government requested - on advice of the Health Council - the RIV to produce IPV itself and the Health Care Inspectorate was solicited to organize vaccination campaigns intending to vaccinate all children in the Netherlands. This mass vaccination against polio was the start of the National Immunization Programme (NIP). Initially, vaccination was provided against diphtheria, whooping cough, tetanus and polio (DTP-IPV). In the 1970-1980s, the program was extended to also provide protection against measles, mumps, and German measles (rubella) (MMR). Since the 1990s, vaccinations against hepatitis B, Haemophilus influenzae type b (Hib), meningococcus C and pneumococci have been included.

Figure 1. Annual number of deaths due to pertussis in the Netherlands 1905-1977 (upper figure), 1978-2008 (lower figure).
Already before introduction of vaccination the mortality of pertussis decreased (Figure 1), presumably due to the higher standard of living, smaller families, improved nutritional status, and the effective treatment of pulmonary complications with antibiotics [13, 17]. In the first years after introduction of routine vaccination, not only the mortality of pertussis decreased but also the case-lethality (i.e., mortality per pertussis case) [13]. In the 1960s and 1970s, the number of deaths decreased to less than 5 per year [18].

As pertussis became rarer in many countries, the need for pertussis vaccination was questioned and the attention shifted from the disease to the adverse events that sometimes follow vaccination. In several countries, suspicions arose that whole-cell pertussis vaccines could very rarely cause serious neurological complications, such as encephalopathy or even death. In Japan, whole-cell pertussis vaccination was eliminated in the end of the 1970s after two infants died within 24 hours of receiving DTP vaccine. In Sweden, after 20 years of vaccination, the efficacy and safety of the vaccine were questioned because pertussis still occurred in vaccinated children and some neurological events after vaccination were blamed on the vaccine. Subsequently, Sweden banned the use of whole-cell vaccination in 1979. In the UK vaccine uptake dropped from 80% in the early 1970s to 30% in 1975 after the public confidence in pertussis vaccination collapsed following reports linking the vaccine with brain damage [19]. Although investigations could not find a causal link between whole-cell pertussis vaccines and severe events, concerns about safety led to the development of acellular pertussis vaccines in the 1970s [20, 21]. Acellular vaccines consist of up to five specific purified *B. pertussis* antigens (pertussis toxin, filamentous haemagglutinin, pertactin, and two fimbrial antigens) and were tested to be non-inferior to whole-cell vaccines but caused fewer side-effects [22].

In the Netherlands in 1976-1984, because of professional and public anxiety concerning the side effects of whole-cell vaccines, the potency of the Dutch vaccine was reduced [23]. Although the vaccine coverage remained high, the number of patients with pertussis increased in the beginning of the 1980s [24]. At that time, it was not clear whether the increased incidence was related to the lower potency of the vaccine or to increased awareness, the establishment of a mandatory notification system or the development of serologic methods for diagnosis. However, later studies showed that the reduction in vaccine potency was followed by significant changes in the *B. pertussis* population, suggesting changes in host immunity. Thus, the increase in notifications may at least in part reflect a true epidemic [25]. In 1989-1994, the disease was endemic in childhood with 4-yearly peaks and most severe disease in infants [26].
Recent developments in the epidemiology of pertussis

Despite more than half a century of vaccination, pertussis is - from all vaccine preventable diseases for which a vaccine was licensed before 1980 - the disease with relatively the lowest reduction in number of cases [14]. Globally, approximately 300,000 children still die from pertussis each year especially in developing countries [27, 28].

Pertussis vaccination helps to prevent disease, but infection may still occur. In addition, neither vaccination nor natural infection provides lifelong protection. Due to waning of vaccine-induced and natural immunity, reinfection may occur and circulation of the bacterium can continue. The severity of the symptoms is affected by the patients’ age, clinical condition, and previous exposure to the organism (either by vaccination or prior infection). Consequently, pertussis infection nowadays has a wide spectrum of clinical manifestations varying from very severe in unvaccinated infants to mild or even asymptomatic in vaccinated children and adults.

In 1996-1997, an outbreak of pertussis occurred in all age groups in the Netherlands especially among vaccinated patients [29]. Changes in vaccination coverage, in diagnostic procedures, in notification practice, and interference from other vaccinations, could not explain the epidemic [29, 30]. The sudden increase among vaccinated children in combination with observed changes in the *B. pertussis* population supported the hypothesis that, due to antigenic changes, circulating strains of *B. pertussis* had become less sensitive to vaccine-induced immunity [30-32]. The effect of antigenic changes may have been aggravated by the low immunogenicity profile of the Dutch whole-cell vaccine used at that time. Since the sudden upsurge in 1996-1997, the incidence of reported pertussis cases has remained high with peaks every 2-3 years (Figure 2).

A number of other countries also experienced a resurgence of pertussis in the last decade, especially among adolescents and adults [2, 3, 33-35].

![Figure 2. Monthly number of cases notified for pertussis in the Netherlands 1989-2009.](image-url)
Due to the re-emergence of pertussis the vaccination strategy for pertussis has changed in the Netherlands. Until 1999, children were vaccinated at the age of 3, 4, 5, and 11 months, and after 1999 this schedule was enhanced by decreasing the age for the first vaccinations to 2, 3, 4, and 11 months [36, 37]. Because of the high incidence in 5-9-year-olds, in November 2001 an acellular booster vaccination for four-year-olds was introduced in the NIP [38]. Because of concerns on side-effects and effectivity, the Dutch whole-cell vaccine was replaced by an acellular vaccine in 2005 [39]. Recent changes regarding pertussis vaccination in the NIP are summarized in Table 1.

Since healthy children are exposed to vaccinations recommended by the government, it is a governmental responsibility to monitor the effectiveness, safety, and reliability of (changes in) the nationwide vaccination programme. The evaluation of the NIP consists of five pillars involving surveillance of: disease incidence, immune status, the pathogen population, safety, and vaccination coverage [40]. Whereby surveillance is defined as: the ongoing systematic collection and analysis of data and the provision of information which leads to action being taken to prevent and control a disease [41].

**Table 1. Recent changes regarding pertussis vaccination in the Netherlands.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997 (November)</td>
<td>Introduction of a ‘stronger’ whole-cell vaccine into the National Immunization Programme, i.e. the criteria for release of the vaccine were enhanced, in that lots were required to contain at least seven International Units (IU) instead of 4 IU.</td>
</tr>
<tr>
<td>1999 (January)</td>
<td>Acceleration of vaccine schedule from 3, 4, 5, 11 months after birth to 2, 3, 4, 11 months after birth.</td>
</tr>
<tr>
<td>2003 (March)</td>
<td>Introduction of combined vaccine DTwP-IPV-Hib at 2, 3, 4, and 11 months, for birth cohorts from 1st April 2002 onwards.</td>
</tr>
<tr>
<td>2005 (January)</td>
<td>Replacement of DTwP-IPV-Hib (NVI) with DTaP-IPV-Hib (Infanrix IPV-Hib (GSK)) at 2, 3, 4, and 11 months, for birth cohorts from 1st February 2004 onwards.</td>
</tr>
<tr>
<td>2006 (January)</td>
<td>Replacement of Infanrix IPV-Hib (GSK) by Pediacel (SP MSD) at 2, 3, 4, and 11 months, for birth cohorts from 1st February 2005 onwards.</td>
</tr>
<tr>
<td>2006 (July)</td>
<td>Introduction of combined DTaP-IPV vaccine (Triaxis Polio, SP) for children at 4 years of age born from July/August 2002 onwards.</td>
</tr>
<tr>
<td>2008 (January)</td>
<td>Replacement of Pediacel (SP MSD) by Infanrix IPV-Hib (GSK) at 2, 3, 4, and 11 months. Infanrix IPV (GSK) also available for 4-year-olds.</td>
</tr>
</tbody>
</table>
Outline of the thesis
The main objectives of the studies described in this thesis are to explain trends in the epidemiology of pertussis in the Netherlands in the past decade, and to guide policy and development of control strategies for pertussis in the Netherlands. The studies in this thesis are predominantly based on disease surveillance, a method used to give a description of the frequency and distribution of the disease. Disease surveillance for pertussis in the Netherlands relies on mandatory notifications, laboratory reports, GP sentinel registrations, death registrations, and hospital discharge diagnoses from the National Medical Register. Besides studying trends in the prevalence of pertussis, based on the reported number of pertussis cases in infants, a household study was conducted to investigate transmission routes for pertussis. However, since pertussis infections can be mild or asymptomatic and consultation of a general practitioner is often not required, these clinical surveillance sources underestimate the true number of pertussis infections. To provide insight into the prevalence of clinical disease as well as subclinical infections we used immunosurveillance, which is the assessment of specific antibodies in serum to indicate exposure to a pathogen by vaccination or natural infection.

Surveillance of the vaccination coverage was used to estimate vaccine effectiveness. Nowadays, the coverage for pertussis vaccination is circa 96% in infancy and circa 90% for the preschool booster [42]. Finally, since the introduction of vaccination may force pathogens to adapt towards a phenotype that best fits its present environment [43, 44], we attempted to interpret clinical surveillance data in relation to changes observed in phenotypic or genotypic characteristics of B. pertussis (pathogen surveillance).

This thesis is divided in four parts. In the first part (chapters 2 to 4) we describe the disease burden of pertussis. Chapter 2 comprises a study of the impact of the introduction of the preschool booster. In chapter 3, we evaluate the burden of disease in monetary terms, and in chapter 4 the association between pertussis in infancy and health outcomes on toddler age is studied. In the second part (chapters 5 and 6), we estimate the infection frequency of pertussis, based on serological studies in pregnant women (chapter 5), and more widely in the general population (chapter 6). The third part of this thesis (chapters 7 and 8) comprises studies on transmission routes for pertussis. In chapter 7, transmission routes are studied by looking at age-specific seasonal trends in the occurrence of pertussis, and chapter 8 describes the results of a household study on the transmission of pertussis to infants. Finally, in view of future vaccination strategies, we discuss the possibilities of maternal vaccination to prevent pertussis in infancy (chapter 9), and we consider ways to optimize protection of the population against pertussis (chapter 10, general discussion).
All studies in this thesis were performed within the Centre for Infectious Disease Control of the RIVM and represent a collaboration between the Epidemiology and Surveillance unit and the Laboratory for Infectious diseases and Screening.

References

The Chinese name for pertussis is '100-day cough' which refers to the characteristic prolonged period of coughing.

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Impact of acellular pertussis preschool booster vaccination on disease burden of pertussis in the Netherlands
Abstract

Background: An acellular preschool booster vaccination against pertussis has been included in the National Immunization Programme in the Netherlands, since November 2001. We studied the impact of this preschool booster on the epidemiology of pertussis.

Methods: We analysed and compared pertussis cases registered in the national notification system, hospital registry, and death registry between the periods 1998-2001 (without preschool booster) and 2002-2005 (with preschool booster).

Results: In 2002-2005, the incidence of hospitalisations and notifications in 1-4-year-olds were 48% and 44%, lower respectively, than in 1998-2001. Similarly, in 5-9-year-olds the incidence of hospitalisations and notifications had decreased 32% and 15%, respectively.

In 2005, vaccine effectiveness for the preschool booster among children born between January 1, 1998 and January 1, 2001 – all of whom had been eligible for the booster – was estimated at 79% (95%CI: 71-85). In infants aged 0-6 months, the incidence of hospitalisations per 100,000 population decreased 40%, from 222.5 to 133.6. In contrast, among cohorts aged 10-19, 20-59, and ≥ 60 years, the incidence of notifications increased 60%, 44%, and 68%, respectively.

Conclusions: The preschool booster strongly decreased the disease burden in the targeted cohorts. Importantly, the incidence in infants 0-6 months also showed a decline after introduction of the preschool booster, suggesting reduced transmission from siblings to young infants. Meanwhile, the number of pertussis cases in adolescents and adults increased. With prevention of severe pertussis among infants as focus, this effect should not be ignored in the discussion on future vaccination strategies for pertussis.
Introduction
Vaccination against pertussis has resulted in an enormous decrease of the disease incidence and above all of disease severity, especially in developed countries [1]. Despite high vaccine coverage (ca. 96%), surveillance sources revealed an outbreak of pertussis among mostly vaccinated children in the Netherlands in 1996-1997 [2]. Changes in vaccination coverage, in diagnostic procedures, in notification practice, and interference with other vaccinations could not explain the epidemic. The sudden increase among vaccinated children in combination with changes in the Bordetella pertussis population and the low immunogenicity profile of the Dutch whole-cell vaccine used at that time supported the hypothesis that, because of antigenic changes, B. pertussis was less affected by vaccine-derived immunity [2, 3]. A number of other countries also experienced a resurgence of pertussis in the last decade, especially among adolescents and adults [4-6]. Although these increases might be partly the result of improved recognition and surveillance, the trend is worrisome. Indeed, many recent studies suggest that the increased circulation of B. pertussis among adults and adolescents has led to increased morbidity and mortality in infants too young to be (fully) vaccinated [7, 8]. Unvaccinated infants are at greatest risk for severe complications or death as a result of pertussis. Because in most countries the first pertussis vaccinations are given after the age of 2 months, protection in this period relies on (incomplete) herd-immunity [9]. Different strategies have been considered to complement or improve current vaccination schedules resulting in better protection of these young infants: maternal immunization [10], universal adult or adolescent immunization, selective immunization of close family members of newborns, selective immunization of child-care workers, reinforcement of the current strategy by enhancing vaccine coverage, and a preschool booster at 4-6 years of age [11].

Because of the high incidence of pertussis among school children in the Netherlands [2] the latter strategy, an acellular booster dose in the year a child turns 4 years of age, has been included in the National Immunization Programme (NIP) since the end of 2001. Other countries that have introduced a preschool booster, report a decreasing incidence in the age groups targeted for the booster vaccination [12, 13]. However, as yet little is known about the duration of protection from the preschool booster against disease and its effectiveness in reducing overall pertussis morbidity through herd-immunity.

To assess the impact of the introduction of a preschool booster on the incidence of pertussis, we studied age-specific trends in pertussis epidemiology in the Netherlands in the period 1998-2001 (when no preschool booster was given) and 2002-2005 (when an acellular pertussis preschool booster vaccination was given). The current study extends a previous report on the routine surveillance data from pertussis in the Netherlands covering the period from 1976-1997 [2].
Methods

Case reporting

Since 1976, notification of pertussis to the Health Care Inspectorate has been obligatory by law in the Netherlands. Disease notification data are presented by the date of onset for the period January 1, 1998 to December 31, 2005.

Since 1988, the case definition for notification includes a clinical picture compatible with pertussis (i.e., serious cough with a duration of more than 2 weeks, coughing attacks, or cough followed by vomiting) in combination with: isolation of *B. pertussis* or *B. parapertussis*, detection of *B. pertussis* or *B. parapertussis* DNA by polymerase chain reaction (PCR), or a significant rise in IgG antibodies against pertussis toxin (Ptx) or IgA antibodies against whole cell sonicate of *B. pertussis* in paired serum samples, or a single serum sample with IgA/IgG-Ptx titres above a defined age-specific cut-off value [14], or contact in the last 3 weeks with a laboratory confirmed patient with *B. pertussis* or *B. parapertussis* infection. PCR as a method of confirmation for notification has been accepted since 1997. This case definition has not changed during the period of study. Since 1998, the percentage of infants ≤1 year in whom pertussis was confirmed by PCR or culture varied between 30% and 45%. In children >1 year and adults, about 5% of the pertussis cases were confirmed by PCR or culture. In the remaining patients pertussis was serologically confirmed. These percentages have not changed during the period of study. Fewer than 5% of PCR or culture confirmed cases are *B. parapertussis* positive in the Netherlands [15]. More detailed information regarding the notification system for pertussis can be obtained from de Greeff et al. [16].

Hospital admissions and deaths

Hospital episodes of pertussis from January 1998 through December 2005 were extracted from the National Medical Register. Diagnoses were recorded using the 9th International Classification of Diseases based on clinical diagnoses. Cases with code 0330 (Whooping cough caused by *B. pertussis*), 0331 (Whooping cough caused by *B. parapertussis*), 0338 (Whooping cough caused by other specified organism), or 0339 (Whooping cough caused by unspecified organism) as main discharge diagnosis were included. The annual number of deaths in the period of study caused by pertussis (ICD-10 code A370, A371, A378, and A379) was obtained from Central Statistics in the Netherlands.

Vaccine schedule and vaccines used

In the Netherlands, nationwide pertussis vaccination has been implemented in the NIP since 1957. Until 1999, vaccination with a combined diphtheria, tetanus, pertussis and inactivated polio vaccine (DTP-IPV) was given at 3, 4, 5, and 11 months of age. Since 1999, this schedule
has been accelerated and nowadays children are vaccinated at 2, 3, 4, and 11 months. Until
2004, a nationally manufactured whole-cell vaccine was used as pertussis component of DTP-
IPV for the primary series. In 1997, small changes in the production process of the vaccine in
the Netherlands were implemented, resulting in a higher protection as measured in the release
test in mice, and in an increased consistency of the production process. The whole cell vaccine
was replaced by a 3-component vaccine (Infanrix-IPV-HIB, from GSK) in 2005, followed by
a 5-component acellular vaccine (Pediacel; Sanofi, Val de Reuil, France) in 2006.
For the preschool booster vaccination introduced in 2001, a 3-component acellular vaccine
was used (by GSK), which was given concomitantly, but as a separate injection, with DT-IPV.
During the period under study in this article, children who were eligible for the preschool
booster could according to the NIP only have been primed with a whole-cell vaccine.

Statistical analyses
The number of notifications, hospital admissions, and deaths as a result of pertussis per year,
per month, and per age group were extracted from the different registrations. To calculate
annual incidence rates/100,000, the total number of cases for a year was divided by the total
population on the first of January of the relevant year. To study the effect of the preschool
booster, average incidence rates in the 4 years before (1998-2001) and 4 years after (2002-
2005) introduction of the preschool booster were compared. In this way, the effect of year to
year fluctuations was minimized.
Age-specific average incidence rates in both periods were compared as incidence rate ratios
(relative risks or RR) with 95% confidence intervals (95%CI). Vaccine-effectiveness (VE)
for vaccination in age groups targeted for the fifth dose, were estimated from notifications
according to the screenings method, whereby estimates were derived using the equation:

$$VE(\%) = 1 - \frac{PCV}{(1-PCV)} \times \frac{(1-PPV)}{PPV},$$  \[17\]

where PCV is the proportion of notified cases that has been vaccinated and PPV the proportion
of the population that has been vaccinated. The PPV was assumed to be 93% for the preschool
booster [18]. Incompletely vaccinated cases were excluded from the estimation, 95%
confidence intervals were calculated as described in Hightower et al. [19]. Calculations were
performed using SAS version 9.1, Excel, and Episheet [20].
Results

Annual incidence rate

In the period 1998-2005, the annual incidence rate of pertussis notifications varied between 16.0 in 1998 and 59.8 in 2004 and for the hospitalisations between 0.9 in 2003 and 3.0 in 1999 (Figure 1). Every 2-3 years, an increase in both incidence rates took place, with peaks reported in 1999 (notifications 44.3, hospitalisations 3.0), 2001 (notifications 50.2, hospitalisations 2.3), and 2004 (notifications 59.8, hospitalisations 1.8) (Figure 1). Notifications for pertussis showed an increasing trend, whereas for the hospitalisations a decreasing trend in pertussis incidence was observed.

From 1998 until 2005, 5 deaths as a result of pertussis were reported: 1 in 1998, 3 in 1999, and 1 in 2004, all were children <3 months of age.

The overall incidence rate in the period when no preschool booster was given (1998-2001) compared with the period when the preschool booster was included in the NIP (2002-2005), was almost similar for notifications (34.3 versus 35.3, respectively), whereas for hospitalisations the incidence rate decreased (2.1 versus 1.4, respectively).

Age-specific incidence rates

In agreement with the overall trends, peaks in notifications occurred for all age groups in 1999, 2001, and 2004 (Figure 2). The peaks of notified cases among cohorts aged 0-year and 5-9 years remained almost equally high. In contrast, the height of the peaks among 1-4-year-olds decreased and among adolescents (10-19 years) and adults (20-59 years and ≥60 years) a significant increase was seen (Figure 2).

Figure 1. Yearly incidence of notified pertussis cases (bars) and hospitalised pertussis cases (line) in 1998-2005.
Furthermore, for hospitalisations in infants and children <10 years of age, the height of the peak in 2004 was lower than in 2001, whereas among the older children and adults the peak in 2004 was higher than in 2001 (Figure 2).

In Table 1, age-specific incidence rates for hospitalisations and notifications per 100,000 population in the period without (1998-2001) and with (2002-2005) a preschool booster are presented. For comparison of both periods, incidence rate ratios (relative risks) are presented. Among 1-4 and 5-9 years old cohorts − both of which contain individuals eligible for the booster − the RR for hospitalisations in the period when a preschool booster was given

Figure 2. Annual incidence/100,000 of hospitalisations on left axis (line) and notified pertussis cases on right axis (dotted line) by age category in 1998-2005.
were 0.52 (95%CI: 0.41-0.66) and 0.68 (95%CI: 0.49-0.94), respectively (Table 1). This corresponds with a decrease of hospitalisations among 1-4-year and 5-9-year-olds of 48% and 32%, respectively, in the booster period. Similarly, the incidence rate for notifications among 1-4 and 5-9-year-old cohorts decreased with, respectively, 44% and 15% between both periods (Table 1).

The relative risk for hospitalisations and notifications in 0-5-months-old infants amounted to 0.60 (95%CI: 0.54-0.67) and 0.80 (95%CI: 0.71-0.89), respectively. Thus, the incidence rates of hospitalisations and notifications among infants <6 months of age were 40% and 20%, respectively, lower in the period when a preschool booster was included (Table 1). This decrease in incidence rates was seen for both unvaccinated as well as partly vaccinated infants <6 months of age. Compared with the period before introduction of the booster, the incidence rates of hospitalisations for the 0-1 month, 2-3 month, and 4-5 month age group decreased with 44%, 35%, and 42%, respectively.

To rule out the effect of the replacement of the Dutch whole cell vaccine by an acellular vaccine for the primary series in 2005 in our calculations, we also compared incidence rates in the period 1998-2001 with 2002-2004. This comparison still revealed a 37% reduction (RR=0.63, 95%CI: 0.56-0.70) in hospitalisations and 19% reduction (RR=0.81, 95%CI: 0.71-0.91) in notifications in infants <6 months of age.

Table 1. Age-specific incidence rates per 100,000 population for pertussis hospitalisations and notifications in 1998-2001 and 2002-2005, and relative risk (95%CI) for comparison of the incidence rates between both periods.

<table>
<thead>
<tr>
<th>age group</th>
<th>1998-2001</th>
<th>2002-2005</th>
<th>relative risk (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hospitalisations</td>
<td>notifications</td>
<td>hospitalisations</td>
</tr>
<tr>
<td>0-5 months</td>
<td>222.5</td>
<td>166.1</td>
<td>133.6</td>
</tr>
<tr>
<td>6-11 months</td>
<td>26.5</td>
<td>82.4</td>
<td>30.2</td>
</tr>
<tr>
<td>1-4 years</td>
<td>6.5</td>
<td>153.8</td>
<td>3.4</td>
</tr>
<tr>
<td>5-9 years</td>
<td>2.3</td>
<td>199.0</td>
<td>1.6</td>
</tr>
<tr>
<td>10-19 years</td>
<td>0.3</td>
<td>42.6</td>
<td>0.3</td>
</tr>
<tr>
<td>20-59 years</td>
<td>0.1</td>
<td>10.9</td>
<td>0.1</td>
</tr>
<tr>
<td>≥ 60 years</td>
<td>0.1</td>
<td>7.0</td>
<td>0.1</td>
</tr>
<tr>
<td>total</td>
<td>2.1</td>
<td>34.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>
In contrast, among the 10-19, 20-59, and over 60-year-olds, the incidence rates of both hospitalisations and notifications were substantially higher in the periods 2002-2005 than in 1998-2001. Between both periods the incidence of hospitalisations among 10-19, 20-59, and over 60-year-olds had increased 4%, 9%, and 37%, respectively (Table 1). During the same periods, the incidence of notifications in these age groups had increased 60%, 44%, and 68%, respectively.

**Vaccine-effectiveness**

VE for the preschool booster among children born between January 1, 1998 and January 1, 2001 — all of whom had been eligible for the booster — was estimated in 2005 at 79% (95%CI: 71-85). For the first (year of birth 1998), second (year of birth 1999) and third (year of birth 2000) cohort who became eligible for the booster, the VE in 2005 amounted to 73% (95%CI: 40-88), 74% (95%CI: 54-86) and 84% (95%CI: 75-90), respectively.

**Discussion**

Despite a high vaccination coverage in infancy (>96%), pertussis is still endemic in the Netherlands. After the sudden upsurge in 1996-1997, the incidence of pertussis has remained significantly higher than in the period before 1996 [2], with peak rates observed every 2-3 years [21]. The introduction of the preschool booster vaccination for 4-year-olds with an acellular vaccine in 2001 caused a significant decrease in the incidence of pertussis among the targeted population. No evidence was found for waning immunity 4 years after the preschool booster as the estimated VE remained high, 73%-84%, in the vaccinated cohort. The observed decline in the incidence among young infants suggests that the preschool booster has indirectly protected these susceptible infants. By contrast, in the same period the incidence among adolescents and adults increased.

Before introduction of the preschool booster in 2001, peak incidences for notified cases occurred among unvaccinated or incompletely vaccinated infants <6 months of age and among schoolchildren aged 4-5 years [2]. Apparently, as a result of waning vaccine-derived immunity a substantial number of vaccinated children were again susceptible to pertussis before school entry. Because acellular vaccines were expected to cause fewer side effects and to be more efficacious, the Dutch whole-cell vaccine was replaced by an acellular vaccine in 2005. The expectation is that this will delay the waning of vaccine-derived immunity. Nevertheless, Swedish studies have shown that immunity induced by acellular vaccines in infancy is still limited to about 5-7 years [22, 23].
One important finding of our study was the beneficial effect of the preschool booster on the incidence of pertussis in infants. In Australia, a fifth dose at 4 years of age has been recommended since 1994. Although high coverage was achieved, a downward trend was seen only in the notification rate of the targeted age group [12]. In fact, the United States reported an increase in morbidity [7] and mortality [8] among infants, despite the use of an acellular preschool booster since 1997. Similarly in New Zealand, an increase in infant pertussis was seen despite 4 doses in infancy and 1 at preschool age [24]. Based on mathematical modelling, Hviid et al. [25] estimated that a preschool booster could prevent 18% of hospitalisations in infancy, but our study suggests that a much higher proportion of severe disease is prevented by the preschool booster. Indeed, the incidence of severe disease, resulting in hospitalisation, among infants less than 6 months of age decreased 40% between 1998-2001 and 2002-2005. Part of the reduction of pertussis among infants might be attributable to the replacement of the whole cell vaccine with a more effective acellular vaccine in 2005. However, analysis of a period when only the whole cell vaccine was used still revealed 37% reduction in hospitalisations in infants less than 6 months of age. This finding suggests that in the Netherlands transmission from siblings to susceptible infants may have been reduced as a result of the preschool booster. Such a herd-immunity effect was not observed for infants aged 6-11 months. Although this may be because of different contact patterns of this age group, the interpretation is also difficult because of the small numbers of infants involved. The accelerated vaccination schedule in place since 1999 could also have contributed to the decreased incidence in infants less than six months. Especially the ≥2-month-olds would have benefited from this. However, as similar decreases in hospitalisations are seen in the <2-month-olds (i.e., the unvaccinated age group), the herd-immunity effect caused by the preschool booster seems to be the most important factor. It may be anticipated that the incidence in the 6-11 months age group will decline more after the introduction of the acellular vaccine in 2005 [26].

It seemed that siblings used to be important sources of infection in infants in the Netherlands. This does not correspond with findings from other studies which found that in regions with a low vaccination coverage children were the main source of infection in infants, whereas in high coverage regions – as in the Netherlands – adults played a relatively larger role [27, 28]. Perhaps, lower immunogenicity of the Dutch whole-cell vaccine since the 1990s combined with changes in the pathogen in that period have reduced the functional vaccine coverage. The reduction in infant morbidity might have been more pronounced if morbidity in adults had not increased [29, 30]. An increase in the number of adolescents and adults with pertussis has also been seen in other countries in Europe [4], the United States [5], Canada [6], and Australia [12]. Estimations of infection frequencies with \textit{B. pertussis}, irrespective of clinical course, have shown that infections in adolescents and adults are substantially higher than reported
in notification systems [31]. This increased incidence in countries with continuously high vaccination coverage is often attributed to better case reporting as a result of more sensitive diagnostic methods or increased awareness of pertussis in these groups. It is important to acknowledge these artefacts in our results. Although PCR as method of diagnosis has partly replaced culture, still only approximately 5\% of notifications are confirmed by PCR in the Netherlands, the rest by serology, and these proportions have not changed recently. The narrowed gap between the incidence based on notifications and hospitalisations in infants in the more recent period suggests that a higher proportion of hospitalised cases is being notified. From general practitioner sentinel stations we know that increased alertness has led to a larger proportion of patients being identified (data not shown), both adults and children. Increased alertness may have partly affected increasing trends in adolescents and adults. However, the observation that epidemiological trends in hospitalisations equal the trends in notifications makes it plausible that changes in the number of notified cases are true changes even though the extent may have been affected by more reliable notification practice.

The increased incidence among adolescents and adults might be partly attributed to waning of vaccine-induced immunity [9, 32, 33]. In addition pathogen adaptation may play a role, as antigenic divergence between vaccine strains and circulating strains has been observed [3, 34]. Several independent studies have shown that strain variation affects vaccine efficacy in the mouse model [35, 36]. Recently vaccinated children are well protected, but this mismatch may shorten the period in which reinfection can occur after vaccination.

Modelling studies have shown that booster vaccinations for adults and adolescents are promising approaches to reduce overall morbidity and mortality of pertussis [37]. The true impact of these strategies on disease burden in infancy depends on accessibility of the target population and transmission patterns between age groups, which apparently differ among countries. Because vaccines do not offer lifelong protection as a result of waning immunity, transmission from adolescents or adults to infants takes place unless boosting is repeated [38]. It might be more effective in the long term to protect young infants earlier in life, by vaccination at birth or through maternal antibodies induced by vaccination of the mother during pregnancy [10]. With prevention of pertussis among infants as focus, the relative and country-specific importance of the sources of infection for these infants (e.g., adults, siblings) needs to be determined. These data can be used to assess, by dynamic modelling, the most effective vaccination strategy to prevent pertussis in infants.
References

Economic analysis of pertussis illness in the Dutch population: implications for current and future vaccination strategies

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Danielle M. van den Heuvel
Frits R. Mooi
Hester E. de Melker

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Abstract

To reduce pertussis disease burden, new vaccination strategies are considered in many countries. Since not only health benefits but also economical aspects play a role when introducing new vaccinations, we estimated medical costs of pertussis in the Netherlands. Besides, we retrospectively performed a cost-utility analysis of the preschool booster introduced in 2001. Our results show that annual costs for pertussis are still considerable (approximately €1.77 million for a population of 16 million). Although infants represented only 5% of cases, they accounted for 50% of the total costs. Hence, the economic burden of pertussis is largely determined by costs per infant case (€1,491) and only to a limited degree by costs per patient in other age groups (circa €75). Despite a substantial reduction in the number of cases, the preschool booster was not considered cost-effective.

The effectiveness of universal adolescent or adult booster strategies – to prevent pertussis in infants – should also be considered from an economical point of view before being implemented.
Introduction
As in many industrialized countries [1-3] pertussis is endemic in the Netherlands, despite high vaccination coverage in infancy (approximately 96%) for over fifty years. Vaccination in childhood protects against severe disease, however, due to waning vaccine induced immunity and possible pathogen adaptation [4] *Bordetella pertussis* continues to circulate and an increasing incidence of pertussis in adolescents and adults has been observed in recent years [3, 5-8]. In many countries pertussis is a mandatory notifiable disease. However, as pertussis in adolescents and adults is often clinically not recognized, the true number of adolescent and adult patients is likely to exceed the notified number [9]. Adults appear to be an important source of severe infection of pertussis in infants [10-12]. To prevent pertussis infection in infants, and to reduce the disease burden in adolescents and adults, many countries are exploring the effectiveness of extending current childhood vaccination programmes to target also adolescents and (specific groups of) adults [13].

For governmental policymakers not only health benefits, but also economic aspects are considered when setting priorities for the introduction of new vaccinations in a National Immunization Programme (NIP). Economic evaluations of pertussis vaccination are often strongly affected by assumptions on the amount of unreported patients and lack of reliable input data [14-19]. Although studies focusing on costs associated with hospitalisations [20], nosocomial outbreaks [21, 22], and specific subgroups [23, 24] are available, no study includes all direct costs caused by pertussis infections in the general population.

This study aims to describe age-specific health care utilisation and costs associated with pertussis in the Netherlands, taking into account costs for patients who are not registered in the routine notification system. Furthermore, we retrospectively evaluated the cost-utility of the preschool booster vaccination introduced in the Netherlands in the end of 2001 [6]. These data are essential for the decision making process regarding prospective vaccination strategies against pertussis.

Methods

*Study population and disease burden*

The number of patients with pertussis in the Netherlands in the period 1998-2005 was estimated from two patient registries: the mandatory notification system and the continuous morbidity registration (CMR). The case definition for mandatory notification includes a clinical picture compatible with pertussis (i.e., serious cough with a duration of more than two weeks and/or coughing attacks and/or cough followed by vomiting) in combination with: isolation of *B. pertussis* or *B. parapertussis*; detection of *B. pertussis* or *B. parapertussis* DNA by PCR;
a significant rise in IgG antibodies against pertussis toxin (IgG-Ptx) or IgA antibodies against whole cell sonicate of B. pertussis in paired serum samples; a single serum sample with IgA/ IgG-Ptx titres above a defined age-specific cut-off value [25]; contact in the last three weeks with a patient with laboratory confirmed B. pertussis or B. parapertussis infection. To adjust for underreporting, mandatory notifications were complemented with the number of patients registered in the CMR coordinated by the Netherlands Institute of Primary Health Care (NIVEL). The CMR is a registration of general practitioners (GPs) covering approximately 1% of the Dutch population, a representative sample of the population in terms of age, sex, and degree of urbanization. The GPs in this sentinel network weekly register the number of patients diagnosed with pertussis, divided into laboratory-confirmed (by paired or single sample serology, culture and/or PCR) and clinical cases. Age-specific incidence rates from the CMR were extrapolated for the whole Dutch population (circa 16 million) using the number of inhabitants on 1st January for the corresponding years. In our calculations, we considered the difference between the number of cases in the CMR and the number of notified patients as the number of clinically or underreported cases. The number of deaths in the period of study due to pertussis (ICD-10 code A370, A371, A378, and A379) was obtained from Central Statistics in the Netherlands [26].

**Health care utilisation**

Number of patients hospitalised and the median length (days) of hospital stay was obtained from the National Medical Register, by extracting all patients with main discharge diagnosis pertussis (ICD-9 codes 0330, 0331, 0338 or 0339). We assumed all hospitalised patients were notified. Based on interim results of a household-contact study on sources of pertussis in infants <6 months of age [27], the proportion of infants requiring treatment at an intensive care unit (ICU) was estimated to be 13% with median length of stay of 8 days. As in the same study none of the infected household members above 6 months of age was admitted to an ICU, and since ICU admission is rarely reported in studies covering the more severe cases in adult populations [28, 29], we assumed ICU admission in patients above 6 months was negligible. The number of GP and specialist consultations per pertussis case and the proportion of patients receiving antibiotics or cough medicine was estimated from a previous study on the disease burden of pertussis among 353 children aged 0-9 years, 54 adolescents aged 10-18 years, and 100 adults who were notified for pertussis in the Netherlands in October 1997- January 1998 [30]. This study showed that for notified children aged 0-9 years the percentages of 1, 2, 3, 4, or 5 GP consultations were 3%, 39%, 38%, 19%, 2%, respectively. For adolescents and adults these percentages were 2%, 42%, 41%, 13%, 1%, respectively. Furthermore, it was
shown that 33% of all notified patients aged 0-9 years and 20% of notified cases aged ≥10 years had a single specialist consult in addition to consulting a GP. In 2001-2005, the method of laboratory confirmation was indicated on the notification form. For the preceding years the use of different diagnostics methods was assumed to be similar to the distribution of methods in 2001-2002. Finally, the proportion of notified patients receiving antibiotics in the 0-year-olds, 1-9, 10-19, 20-44, and ≥45-year-olds was 73%, 69%, 45%, 61%, and 56%, respectively.

In the same age groups the proportion receiving cough medicine was 40%, 46%, 41%, 46%, and 44%, respectively.

For hospitalised cases we counted one GP consult as it is common practice in the Netherlands to first consult a GP who – if necessary – refers the patient to the hospital. For clinically or underreported patients we counted – as a conservative estimate – only one GP consultation.

Costs of health care
The economic burden estimates in this study include only direct medical costs, i.e., costs originating from health care resource utilisation. To estimate the cost-utility ratio for the preschool booster the vaccine and administration costs were also included. All costs per unit are presented in Euro 2007 (Table 1).

Data analysis
In view of possible future vaccination strategies, total costs and costs per case are presented for different age groups (<1, 1-9, 10-19, 20-44, and ≥45 years). To address the impact of the acellular pertussis preschool booster vaccination introduced in the end of 2001, we compared the total costs by periods; i.e., 1998-2001 when no preschool booster was given versus years 2002-2005 when the booster was included in the NIP. By clustering of these years, the effect of year to year fluctuations in the occurrence of pertussis was also minimised.

To relate costs for vaccinating all children at preschool age to cost-savings due to less pertussis cases (or less severe illness, needing less health care), costs per case averted were calculated for the targeted age group as: cost of vaccination plus medical costs in the period with booster minus medical costs in the period without booster, divided by the difference in number of pertussis cases between the periods. We assumed a vaccination coverage of 93% [31].

Health gains due to the preschool booster vaccination were combined with costs in a cost-utility analysis and expressed as quality-adjusted life years (QALYs) gained. The health state values (variously called: utilities, preferences, strength of preference, index, weights, or quality of life weights), reflect the relative desirability of the health state and are measured on an interval scale, where 1 refers to full health and 0 refers to death. Health state values for pertussis were derived from a study by Lee et al. applying the time-trade off (TTO) technique [32].
Table 1. Direct medical costs per unit for pertussis, the Netherlands, 2007.

<table>
<thead>
<tr>
<th>Health care resource</th>
<th>Costs (Euro 2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP consult</td>
<td>21.37a</td>
</tr>
<tr>
<td>Specialist consult</td>
<td>63.89ab</td>
</tr>
<tr>
<td>Hospital admission per day</td>
<td>371.15ab</td>
</tr>
<tr>
<td>ICU admission per day</td>
<td>1,781.18a</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>40 per testc</td>
</tr>
<tr>
<td>PCR/Culture</td>
<td>99 per testc</td>
</tr>
<tr>
<td>Antibioticsde</td>
<td></td>
</tr>
<tr>
<td>0 yrs</td>
<td>13.73</td>
</tr>
<tr>
<td>1-9 yrs</td>
<td>13.87</td>
</tr>
<tr>
<td>10-19 yrs</td>
<td>16.45</td>
</tr>
<tr>
<td>≥ 20 yrs</td>
<td>16.45</td>
</tr>
<tr>
<td>Cough medicinedf</td>
<td></td>
</tr>
<tr>
<td>0 yrs</td>
<td>4.86</td>
</tr>
<tr>
<td>1-9 yrs</td>
<td>8.78</td>
</tr>
<tr>
<td>10-19 yrs</td>
<td>9.04</td>
</tr>
<tr>
<td>≥ 20 yrs</td>
<td>9.04</td>
</tr>
<tr>
<td>Vaccinegh</td>
<td>18.30</td>
</tr>
<tr>
<td>Administration costs</td>
<td>6.20</td>
</tr>
</tbody>
</table>

a Reference [45], adjusted to 2007 using Consumer Price Index.

b Weighted average between regional and university hospital.

c Laboratory for Infectious Diseases and Screening (LIS), RIVM.

d Average costs per age group of the medicines recommended in the 2007 guidelines of the Dutch College of General Practitioners (NHG, http://nhg.artsennet.nl/), the Dutch Pharmaceutical review book 2006 (Farmaceutisch Kompas, http://www.fk.cvz.nl/) and disease-specific fact-sheets of the Dutch Centre for Infectious Disease Control (http://www.rivm.nl/cib/infectieziekten/Pertussis/). Doses in children 0-9 years were calculated using the mean bodyweight obtained from age-specific growth curves [46].

e Azithromycin, erythromycin or clarithromycin.

f Noscapin, codeine or promethazin.

g A single acellular vaccine (http://www.fk.cvz.nl/).

h The vaccine was given concomitantly, though as a separate shot, with combined diphtheria, tetanus, and inactivated polio vaccine (DT-IPV) booster.

As a conservative estimate we used the median values of disease health states corresponding to a ‘mild’ health state. Values for ‘infant pertussis’ (0.72) were used for 0-year-olds, values for ‘adolescents’ (0.87) for age groups 1-9 and 10-19 years, and values for ‘adults’ (0.96) for patients aged 20 years and older. For all ages the duration of the health state was eight weeks, corresponding to 8/52 years. Death was calculated as a loss of 4 life years (length of registration period), but we also performed the calculations for the loss of a whole life, using life expectancy at birth in 2005 of 78.8 years and a discount rate of 1.5% (resulting in 46.2 years) [33].
Assuming that the health state with pertussis is \( P \), the total number of QALYs lost over the period before the booster can be calculated as (and correspondingly for period after the booster):

\[
\sum_{1998}^{2001} \text{QALYs lost} = (1 - \text{value}_{\text{state } P}) \times \text{duration of state } P \times \text{number of cases in state } P
\]  

(1)

The cost-utility ratio describing the cost per QALY gained due to vaccination can then be estimated:

\[
\frac{\text{Costs} / \text{QALYs gained}}{\sum \text{QALYs lost} - \sum \text{QALYs lost}} = \frac{\text{Vaccination costs} + \sum \text{(Health care costs)} - \sum \text{(Health care costs)}}{\sum \text{QALYs lost} - \sum \text{QALYs lost}}
\]  

(2)

In the Netherlands, such a ratio is generally considered cost-effective for preventable diseases if below a threshold value of €20,000.

Analyses were performed using SAS version 9.1 and Microsoft Excel.

**Results**

**Disease burden**

Table 2 shows number of patients registered in the notification system and CMR, by age group and period. In 1998-2001, the number of patients with pertussis registered in the CMR was a factor 3.0 higher than in the notification system, in 2002-2005 the number was 1.5 higher. In all age groups the numbers of patients registered in the CMR decreased from 1998-2001 to 2002-2005, with a decrease ranging from 15% (≥45 years) to 65% (0 years).

In the notification system the number of patients decreased among 0-year-olds (13%) and 10-year-olds (26%), while a 40% increase was seen in adults aged 20-45 years and a 68% increase in both 10-19-year-olds and ≥45-year-olds.

Five deaths due to pertussis were reported: one in 1998, three in 1999 and one in 2004, all were children less than 3 months of age.

**Health care utilisation**

In Table 3 the health care consumption for pertussis, per age group and period is given. In the 8 years under study, GPs were on estimate consulted 173 thousand times with complaints diagnosed as pertussis, corresponding to almost 22 thousand GP consultations per year (Table 3). Children aged 1-9 years old accounted for 54% of all consultations, though this percentage
decreased from 59% in 1998-2001 to 46% in 2002-2005. In 1998-2001 and 2002-2005, a total of respectively 20,955 (97%) and 22,233 (97%) notified cases were laboratory confirmed. This suggests that one out of five GP consultations for pertussis led to laboratory confirmation in 1998-2001, and one out of three in 2002-2005.

The number of patients needing hospitalisation also decreased between the two periods. In both periods, infants below 1 year of age accounted for 74% of all hospitalisations. Among 0-year-olds, 1-9-year-olds and 20-44-year-olds, the number of hospitalisations decreased when comparing the two periods, whereas in the other age groups a slight increase was seen. The median length of hospitalisation decreased with 1 day.

Cost of treatment
In the 8 years of study, the direct costs for pertussis were circa €14 million, corresponding to €1.77 million per year (Table 4). Total costs decreased from €8.4 million in 1998-2001 to €5.8 million in 2002-2005. The majority of costs were attributed to hospitalisation including ICU admission. Costs for GP consultations accounted for 26% of all costs in both periods. In general, the absolute and relative contribution of costs for diagnostics, antibiotics and cough medicine were higher in 2002-2005 compared to 1998-2001.

Pertussis in infants was responsible for 51% of total costs in 1998-2001 and 42% of total costs in 2002-2005. The 1-9-year-olds, 10-19-year-olds, 20-44-year-olds and ≥45-year-olds accounted in 1998-2001 for respectively 32%, 7%, 6%, and 4% of the total costs, and in 2002-2005 these percentages were 28%, 12%, 9%, and 8%, respectively. Over the period of study, the estimated mean direct medical costs per clinical case for 0-year-olds, 1-9-year-olds, 10-19-year-olds, 20-44-year-olds, and ≥45-year-olds were €1491, €81, €79, €76, and €77.

Table 2. Number of reported patients with pertussis registered in the CMR and notification system in the Netherlands, by age group and period (1998-2001 versus 2002-2005).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>CMR 1998-2001 absolute number (%)</th>
<th>CMR 2002-2005 absolute number (%)</th>
<th>Notified patients 1998-2001 absolute number (%)</th>
<th>Notified patients 2002-2005 absolute number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3,334 (5)</td>
<td>1,165 (3)</td>
<td>995 (5)</td>
<td>867 (4)</td>
</tr>
<tr>
<td>1-9</td>
<td>36,768 (57)</td>
<td>16,511 (48)</td>
<td>12,731 (59)</td>
<td>9,484 (46)</td>
</tr>
<tr>
<td>10-19</td>
<td>9,707 (15)</td>
<td>6,602 (19)</td>
<td>3,202 (15)</td>
<td>5,367 (21)</td>
</tr>
<tr>
<td>20-44</td>
<td>8,502 (13)</td>
<td>4,923 (14)</td>
<td>2,772 (13)</td>
<td>3,874 (16)</td>
</tr>
<tr>
<td>≥45</td>
<td>5,852 (9)</td>
<td>4,963 (15)</td>
<td>1,982 (9)</td>
<td>3,333 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>64,163</td>
<td>34,164</td>
<td>21,682</td>
<td>22,925</td>
</tr>
</tbody>
</table>
respectively. For the same age groups the mean direct costs per notified case were €3572, €162, €130, €131, €134, respectively.

Economic evaluation of preschool booster

The costs for vaccinating children with a preschool booster amounted to €18.7 million in 2002-2005. Among children aged 1-9 years the costs per case averted due to the preschool booster were estimated at €922. Taking into account that the preschool booster may also have reduced pertussis in infants aged <1 year, the costs per case averted were €830. Equation (2) resulted in a cost-utility ratio for the preschool booster vaccination of €43,463 per QALY gained in children aged 1-9 years in the period of study. Including life years gained in infants aged <1 year would yield a cost-utility ratio of €30,855 per QALY gained, decreasing to €24,724 per QALY gained if calculating loss of expected life years at birth (discounted with 1.5%).

Discussion

To the best of our knowledge this is the first study that attempts to estimate the national burden of pertussis in monetary terms. Our results show that annual costs for pertussis are still considerable (approximately €1.77 million) and do not substantially deviate from those of varicella zoster virus (€1.2 million for varicella and €3.0 million for zoster) [34] for which inclusion in NIP is currently under consideration. As shown before [20, 35, 36], the majority of costs for pertussis are incurred by costs for hospitalisation and infants account for the bulk of these. Thus, despite the high disease burden in both children and adults, the economic burden of pertussis is largely determined by costs per infant case (€1,491) and only to a limited degree by costs per patient in other age groups (circa €75).

Costs per case calculated in our study are lower than reported in previous studies from the US [23, 24]. Although charges for medical consumption differ across countries and exchange rates may fluctuate, hampering direct comparison of costs, the costs per case also depend on the estimated level of underreporting of clinical patients [16]. Acknowledging the fact that the true contribution of underreported or under-diagnosed patients may even be more substantial [9], we think that with inclusion of the CMR estimates our results give a more complete picture of the medical costs of pertussis in the society. First of all, CMR estimates are less likely to be hampered by under recognition since participating GPs are asked to weekly report pertussis and therefore will be more alert for the disease. Secondly, CMR estimates were validated by estimates from other sources: based on an additional questionnaire we know that in 2001-2005 the total number of laboratory confirmed patients in the CMR (45-65%) almost equalled the total number of notified patients in these years (data not shown), justifying our assumption that
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>GP consultations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 yr</td>
<td>1-9 yrs</td>
<td>10-19 yrs</td>
</tr>
<tr>
<td></td>
<td>3,965</td>
<td>59,275</td>
<td>14,986</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>culture/PCR</td>
<td>296</td>
<td>579</td>
<td>76</td>
</tr>
<tr>
<td>paired serum</td>
<td>148</td>
<td>1,601</td>
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<tr>
<td>single serum</td>
<td>514</td>
<td>10,099</td>
<td>2,718</td>
</tr>
<tr>
<td>unknown/epidemiological linked</td>
<td>37</td>
<td>452</td>
<td>100</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>730</td>
<td>8,790</td>
<td>1,451</td>
</tr>
<tr>
<td>Cough medicine</td>
<td>398</td>
<td>5,833</td>
<td>1,301</td>
</tr>
<tr>
<td>Specialist consultations</td>
<td>74</td>
<td>4,104</td>
<td>636</td>
</tr>
<tr>
<td>ICU admission</td>
<td>116</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hospital admission</td>
<td>997</td>
<td>296</td>
<td>22</td>
</tr>
<tr>
<td>Median duration (days)</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
all laboratory confirmed cases in the CMR were notified. Likewise, estimates of the number of laboratory confirmed cases in the CMR corresponded with the number of positive patients according to the diagnostic serology database for pertussis at the RIVM (data not shown).

Remarkably, the number of patients registered in the CMR shows a decreasing trend in recent years, while the number of notified patients has increased, especially among adolescents and adults. We have no full explanation for the conflicting trends in the number of patients reported according to the CMR and notification system. The narrowing gap between the number of patients in the CMR and notifications, suggests improved alertness and/or reporting practice, implying that our estimates of the costs for the period before 2001 underestimate the actual costs for pertussis in that period. On the other hand, the slightly increased number of hospitalisations in adolescents and adults might suggest that part of the increase of notified patients may indicate a real increase of more typical – and probably better recognizable – pertussis disease in this group [2, 7, 8].

Following the trends in disease burden, the costs for GP consultations and diagnostic testing in adolescents and adults have started to contribute more to the total economic burden of pertussis in recent years. In contrast, the absolute and relative contribution of costs for hospitalisation has decreased in the Netherlands. This is partly related to a general tendency to shorten hospitalisations and discharge patients on an earlier stage [26]. The absolute decline in hospitalisations among infants in recent years is most likely due to a herd immunity effect of the preschool booster vaccination [6].

In addition to estimating the economic burden of pertussis we have evaluated the cost-effectiveness of the preschool booster introduced in 2001. Our results show that this acellular preschool booster vaccination was not cost-saving within the framework of the NIP. Recognizing that an intervention does not have to be cost-saving to be worthwhile implementing, we also performed a cost-utility analysis. This showed that the preschool booster was a little over the limit of being cost-effective when taking into account also the observed herd-immunity effect in young infants. However, the quality of life values used might include altruism when parents are asked to value states for children [32], and this may underestimate the value of the health state and overestimate the QALYs gained. Despite this and other reported shortcomings by Lee et al. [32], these values were used in lack of more reliable data. Conversely, it may also be argued that we underestimated the health gain by the preschool booster, when only including the health gain in the four years of study. Edmunds et al., showed by using a dynamic transmission model that a booster vaccination for 4-year-olds could potentially be cost-effective depending particularly on the number of deaths prevented and on the size of the herd-immunity effect in infants and children [36]. Obviously, surveillance has to be continued to monitor the eventual long term effects of the preschool booster.

<table>
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<tbody>
<tr>
<td>GP consultations</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0 yr</td>
<td>1,266,457</td>
<td>320,181</td>
<td>279,469</td>
<td>194,760</td>
<td>2,145,591</td>
<td>36,353</td>
<td>712,842</td>
<td>330,556</td>
<td>244,650</td>
<td>220,869</td>
<td>1,545,270</td>
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<tr>
<td>1-9 yrs</td>
<td>4,722</td>
<td>262,157</td>
<td>40,631</td>
<td>35,239</td>
<td>367,869</td>
<td>5,267</td>
<td>184,358</td>
<td>64,116</td>
<td>46,884</td>
<td>39,156</td>
<td>339,781</td>
</tr>
<tr>
<td>10-19 yrs</td>
<td>396,387</td>
<td>31,548</td>
<td>21,527</td>
<td>20,784</td>
<td>2939,870</td>
<td>1,328,713</td>
<td>168,502</td>
<td>23,382</td>
<td>8,536</td>
<td>26,723</td>
<td>1,555,556</td>
</tr>
<tr>
<td>20-44 yrs</td>
<td>1,650,509</td>
<td></td>
<td></td>
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<tr>
<td>20-44 yrs</td>
<td>1,650,509</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>ICU admission</td>
<td>61,722</td>
<td>589,354</td>
<td>140,921</td>
<td>119,004</td>
<td>988,791</td>
<td>53,128</td>
<td>429,643</td>
<td>235,709</td>
<td>164,609</td>
<td>143,072</td>
<td>1,026,221</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>10,018</td>
<td>121,911</td>
<td>23,867</td>
<td>27,816</td>
<td>201,870</td>
<td>8,730</td>
<td>90,818</td>
<td>38,874</td>
<td>30,704</td>
<td>209,130</td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>1,934</td>
<td>51,217</td>
<td>11,759</td>
<td>11,402</td>
<td>79,024</td>
<td>1,685</td>
<td>38,154</td>
<td>19,710</td>
<td>15,935</td>
<td>13,287</td>
<td>88,772</td>
</tr>
<tr>
<td>Total costs</td>
<td>4,283,255</td>
<td>2,687,482</td>
<td>568,908</td>
<td>494,456</td>
<td>8,388,715</td>
<td>2,424,921</td>
<td>1,624,316</td>
<td>713,539</td>
<td>519,488</td>
<td>473,811</td>
<td>5,756,075</td>
</tr>
</tbody>
</table>
Since recent studies have shown that in the Netherlands pertussis was often diagnosed too late to start antibiotic prophylaxis of family members at high risk [37] and the acellular vaccine was well tolerated [38], costs associated with prophylactic treatment and QALY's lost to negative side effects are likely to be negligible.

We acknowledge there are still uncertainties around our estimates of disease burden and assumptions on health care utilisation. Furthermore, in our calculation of costs we did not include indirect costs (loss of work productivity), while these may add substantially to the overall costs [14, 24, 35]. However, preliminary results from a household study conducted in the Netherlands [27] show that only 11/175 laboratory confirmed adult pertussis cases stayed at home for one or more days because of infection (unpublished data). Still, our results show that costs of pertussis in adolescents and adults are relatively confined and prevention of pertussis in infants will be the most effective way to save expenses. More importantly from a public health point of view, these infants are the ones suffering from the most severe disease sometimes leading to death. Vaccinating adolescents and adults, as often suggested [13, 18, 39], may reduce circulation of \textit{B. pertussis} and hence transmission to vulnerable infants. Due to waning vaccine induced immunity boosting has to be repeated. This would be an expensive strategy of which the (cost) effectiveness is mainly determined by the level of herd-immunity attained, the true incidence and the duration of immunity [16-18, 40]. A recent review suggested that, considering the substantial costs necessary to implement population based vaccination strategies for pertussis, these are unlikely to be cost-effective [41]. We believe it will be more advantageous to focus exclusively on directly preventing transmission to infants, i.e., by vaccinating adults who are in close contact with newborns. Although the (cost) effectiveness still has to be investigated one can hypothesize that costs for vaccinating certain target groups will be lower than for decennial boosting of all adults. Moreover, feasibility of this approach might be better as young parents can be motivated during pre-natal health care visits. For the long term, resources should be used to study the possibilities to protect young infants earlier in life, by vaccination shortly after birth [42, 43] or through maternal antibodies induced by vaccination of the mother during pregnancy [44]. Ultimately, the development of improved pertussis vaccines which induce long term immunity is required to tackle the pertussis problem.
References

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Pertussis in infancy and the association with respiratory and cognitive disorders on toddler age

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Submitted for publication
Abstract

Background: Pertussis in unvaccinated infants can run a severe course and is often accompanied by complications. We studied whether there is an association between pertussis hospitalisation in infancy and, respiratory symptoms, growth and cognitive development in early childhood.

Methods: A group of 89 children aged 13-45 months and hospitalised for laboratory confirmed pertussis within the first six months of their life were compared with 172 age-matched children without a history of pertussis. Multivariate logistic regression analysis was used to estimate risk ratios (RR) with 95% confidence intervals (95%CI) of the association between health outcomes and pertussis in infancy. Weight-for-length and length-for-age z-scores were calculated to investigate growth. Van Wiechen scores were compared to study cognitive development.

Results: Children with a history of pertussis in infancy had more often “asthma symptoms” (RR 2.8, 95%CI: 1.1 – 7.0) on toddler age and were more likely to report “respiratory infections” (RR 3.3, 95%CI: 1.6 – 6.6). A history of pertussis in infancy was associated with significantly lower weight-for-length in the first 40 months of life. No significant differences in cognitive development were found.

Conclusions: We found an association between severe pertussis in infancy and respiratory symptoms on toddler age. The mechanisms that may underlie this association require further investigation.
Introduction
Despite routine vaccination with high coverage, pertussis is still endemic in many countries including the Netherlands [1, 2]. Pertussis in unvaccinated infants can run a severe course and is often accompanied by complications such as hypoxia, apnoea, pneumonia or encephalopathy [3-5]. There is only limited data on possible consequences of severe pertussis in infancy in the long term. Results from several studies conducted in the 1980s suggested that children with a history of whooping cough were more likely to experience respiratory symptoms in childhood, though no differences could be demonstrated in physical examination of lung function indices [6-8]. It has been suggested that pertussis in childhood may result in a higher risk for atopic disorders, such as asthma, later in life [7, 9]. This was refuted by others who explained these results by confounding factors (such as socio-economic status and number of siblings) or by the greater susceptibility for pertussis of children predisposed to respiratory morbidity [7, 10, 11].
Studies from the 1950s describe severe intellectual difficulties or mental deficiencies in children who suffered from pertussis in infancy [12-14]. In later studies it was argued that these effects may be present in children with neurological complications, but are in general absent [15]. Interpretation of these findings is problematic, especially in the present time as most of these studies are outdated and medical care has improved since then. Furthermore, the diagnosis of pertussis in these studies is questionable as inclusion of subjects mainly relied on retrospective ascertainment of a history of whooping cough.
Previously, we conducted a nationwide prospective study to identify who introduced pertussis into the household of infants aged <6 months hospitalised for laboratory confirmed pertussis in the Netherlands [3]. In the current study we aimed to investigate whether there is an association between pertussis hospitalisation in infancy and, respiratory symptoms, growth and cognitive development in infants aged 1-3 years.

Methods
Participants
In 2009, children born between July 2005 and February 2008, and hospitalised for laboratory confirmed pertussis (by PCR, culture or serological testing) in infancy (i.e., aged <6 months), were recruited from a previous nationwide study on pertussis in infants [3]. Hospitalisation for pertussis in these infants took place at least 12 months before enrolment in the current study. Based on the Dutch vaccination register, for each child hospitalised for pertussis in infancy three control children were invited from the same postal area and of the same sex and age. None of the controls reported to have had pertussis in infancy.
Data collection

Information on health status of both pertussis and control children, was collected by an extensive questionnaire which was adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) [16].

Four dichotomous health outcomes were defined:

- Mild asthma like symptoms: mucus/sputum production without having a cold, and/or dry cough without having a cold, and/or at least one episode of wheezing or dyspnoea in the past twelve months.
- Respiratory infections: a doctors’ diagnosis of pneumonia above 1 year of age, and/or doctors’ diagnosis of bronchitis in the past in combination with symptoms of or medication for bronchitis in the past twelve months, and/or more than six episodes of severe respiratory symptoms in the past (such as flu, pharyngitis, otitis media or sinusitis).
- Asthma symptoms: more than 4 episodes of wheezing or dyspnoea in the past twelve months, and/or doctors’ diagnosis of asthma in the past in combination with symptoms of or medication for asthma in the past twelve months.
- Skin disorders: itching rash and/or eczema in the past twelve months.

Information on length and weight was obtained from growth curves monitored during routine visits of the child at the Child Health Centre (CHC). Information on cognitive development of participating children was obtained from ‘Van Wiechen developmental investigation form’ routinely collected for each child by CHCs. The Van Wiechen classification scheme is an internationally accepted method to assess motor behaviour, speech, communication, and social skills based on physicians’ observations and parental questioning in order to early detect developmental delays [17]. Two clinicians calculated – independently of each other and blinded from the pertussis history of the child – sum scores on age-specific milestones over the first 15 months of age and of age 15-48 months, based on the number of positively or correctly answered items. Items were grouped to distinguish between language skills and other skills. Sum scores were classified as “good”, “unsatisfactory” or “uncertain”. An unsatisfactory score was given if at least one of the language items, or two items of the other skills, were scored negative. An uncertain score was given if too much data was lacking. In general, 90% of children have a satisfactory score on age-specific milestones.

The study design was approved by the Medical Ethics Committee of the University Medical Centre in Utrecht, and all parents of participating children signed written informed consent.
Statistical analysis
The questionnaires and Van Wiechen scores were analysed with SAS version 9.1, Microsoft Excel, and Episheet [18].
Differences in potential confounding variables (Table 1) between the pertussis group and control group were tested by the χ² test, Fisher’s exact test or Student’s t-test, as appropriate. We considered the study to be a retrospective cohort study and multivariate logistic regression analysis with “modified Poisson” approach [19] was used to estimate risk ratios (RR) with 95% confidence intervals (95%CI) of the association between each outcome and pertussis in infancy. To adjust for potential confounding we fitted multivariate models if there was a significant association between pertussis and the outcome in the univariate model, and included only variables which changed the univariate point estimate of the effect of pertussis with at least 10% [20]. The growth curves of the CHC files were analysed in R. Weight-for-length and length-for-age z-scores were calculated using Dutch references [21]. A z-score, or standard deviation score, is computed to determine the outcome of an individual in relation to reference measurements of a comparable population with the same age and sex. Weight-for-length scores account for individual differences in weight with respect to length, length-for-age scores account for differences in length with respect to age. Z-scores were analysed using a linear mixed effect regression model, where the pertussis group and the control group were compared, adjusted for birth weight, breastfeeding and birth order. Between-child variability was allowed for by including a random slope for length in the weight-for-length analysis, and for age in the length-for-age analysis. We tested if z-scores differed between both groups and whether this difference changed during the period of follow-up. In all analyses, a P-value<.05 was considered statistically significant.

Results
For 89 (57%) of 155 children with a history of pertussis the questionnaire was returned. In the control group, for 172 (37%) of 465 invited children the questionnaire was returned. Characteristics of the pertussis and control group are presented in Table 1. Children in the pertussis group were less frequently completely vaccinated in infancy (i.e., less likely to have received four doses), had more often (older) siblings and were more often living outside the city than control children.
Adjusted for confounding variables, the RR for the association between pertussis and “respiratory infections” amounted to 3.3 (95%CI: 1.6 – 6.6) and for “asthma symptoms” amounted to 2.8 (95%CI: 1.1 – 7.0) (Table 2).
A history of pertussis in infancy resulted in significantly lower weight-for-length in the first 40 months of life, the z-score for the pertussis group differed on average -0.26 (95%CI: -0.51 − -0.01) standard deviations from the control group (P = 0.04). No interaction between group and length was found (P = 0.52), indicating that during this period the pertussis children did not catch up with the control group children. Length-for-age z-scores in the pertussis group differed on average -0.16 (95%CI: -0.41 − 0.09) standard deviations from the control group (P = 0.21). No significant interaction between group and age was found (P = 0.28). Children with a history of pertussis hospitalisation in infancy had more often unsatisfactory language scores, although this difference was not statistically significant (Table 3).

<table>
<thead>
<tr>
<th></th>
<th>Pertussis (n=89)</th>
<th>No pertussis (n=172)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean months (SD)</td>
<td>27 (6.7)</td>
<td>28 (7.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Number of males (%)</td>
<td>42 (47)</td>
<td>86 (50)</td>
<td>0.67</td>
</tr>
<tr>
<td>Birth weight, mean grams (SD)</td>
<td>3,473 (797.5)</td>
<td>3,491 (703.1)</td>
<td>0.85</td>
</tr>
<tr>
<td>Gestation period, mean weeks (SD)</td>
<td>39.1 (2.3)</td>
<td>39.4 (1.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Gestation period &lt;37 weeks (%)</td>
<td>10 (11)</td>
<td>10 (6)</td>
<td>0.12</td>
</tr>
<tr>
<td>Breastfeeding during at least 1 month (%)</td>
<td>56 (63)</td>
<td>120 (70)</td>
<td>0.26</td>
</tr>
<tr>
<td>Completely vaccinated against pertussis (%)</td>
<td>70 (78)</td>
<td>153 (89)</td>
<td>0.03</td>
</tr>
<tr>
<td>Parent(s) of non-western origin (%)</td>
<td>12 (13)</td>
<td>25 (15)</td>
<td>0.82</td>
</tr>
<tr>
<td>Attending child day care (%)</td>
<td>56 (63)</td>
<td>121 (71)</td>
<td>0.22</td>
</tr>
<tr>
<td>Number of siblings in household, mean (SD)</td>
<td>1.9 (1.6)</td>
<td>1.1 (1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>At least one older sibling (%)</td>
<td>73 (82)</td>
<td>97 (56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pets in household (%)</td>
<td>40 (46)</td>
<td>97 (56)</td>
<td>0.11</td>
</tr>
<tr>
<td>Smoking in household (%)</td>
<td>7 (8)</td>
<td>13 (8)</td>
<td>0.93</td>
</tr>
<tr>
<td>Both parents low level of education (%)</td>
<td>11 (12)</td>
<td>16 (9)</td>
<td>0.45</td>
</tr>
<tr>
<td>Family member with allergy (%)</td>
<td>51 (57)</td>
<td>92 (53)</td>
<td>0.55</td>
</tr>
<tr>
<td>Family member with eczema (%)</td>
<td>36 (41)</td>
<td>57 (33)</td>
<td>0.26</td>
</tr>
<tr>
<td>Family member with asthma (%)</td>
<td>22 (25)</td>
<td>28 (16)</td>
<td>0.10</td>
</tr>
<tr>
<td>Living in city (%)</td>
<td>33 (37)</td>
<td>90 (52)</td>
<td>0.02</td>
</tr>
<tr>
<td>History of measles (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>History of varicella (%)</td>
<td>31 (35)</td>
<td>68 (40)</td>
<td>0.56</td>
</tr>
<tr>
<td>Follows special diet (%)</td>
<td>5 (6)</td>
<td>10 (6)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of children with and without a history of pertussis hospitalisation in infancy.
Table 2. Prevalence of respiratory symptoms and skin disorders in children with and without a history of pertussis hospitalisation in infancy.

<table>
<thead>
<tr>
<th></th>
<th>Pertussis (n=86)</th>
<th>No pertussis (n=151)</th>
<th>Crude RR (95%CI)</th>
<th>Adjusted RR a (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild asthma like symptoms (%)</td>
<td>41 (48)</td>
<td>58 (38)</td>
<td>1.3 (0.8 – 1.9)</td>
<td>-</td>
</tr>
<tr>
<td>Respiratory infections (%)</td>
<td>22 (26)</td>
<td>14 (9)</td>
<td>2.8 (1.4 – 5.4)</td>
<td>3.3 (1.6 - 6.6)</td>
</tr>
<tr>
<td>Asthma symptoms (%)</td>
<td>14 (16)</td>
<td>8 (5)</td>
<td>3.1 (1.3 - 7.3)</td>
<td>2.8 (1.1-7.0)</td>
</tr>
<tr>
<td>Skin disorders (%)</td>
<td>20 (23)</td>
<td>35 (23)</td>
<td>1.0 (0.6 - 1.7)</td>
<td>-</td>
</tr>
</tbody>
</table>

a For 3 children in the pertussis group and 28 children in the control group information on confounding variables was missing.

b “Respiratory infections” adjusted for age, sex, and number of siblings; “asthma symptoms” adjusted for age, sex, number of siblings and family member with asthma.

Table 3. Number of children with unsatisfactory scores, by age, and history of pertussis hospitalisation in infancy.

<table>
<thead>
<tr>
<th></th>
<th>Pertussis (n=90)</th>
<th>No pertussis (n=172)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with sufficient data available</td>
<td>No. with unsatisfactory score (%)</td>
<td>No. with sufficient data available</td>
</tr>
<tr>
<td>&lt;15 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language scores</td>
<td>64</td>
<td>2 (3)</td>
<td>124</td>
</tr>
<tr>
<td>Other skills</td>
<td>66</td>
<td>2 (2)</td>
<td>123</td>
</tr>
<tr>
<td>15-48 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language scores</td>
<td>49</td>
<td>9 (18)</td>
<td>106</td>
</tr>
<tr>
<td>Other skills</td>
<td>49</td>
<td>3 (6)</td>
<td>105</td>
</tr>
</tbody>
</table>

Discussion

Adjusted for confounding factors, we showed that children with a history of pertussis in infancy had on toddler age about 3 times higher risk for “respiratory infections” and “asthma symptoms”. A higher risk for respiratory illness in childhood, may be a precursor for asthma in adulthood [22, 23].

Based on the current study we can only speculate on the explanation for the increased risk on respiratory morbidity in children with a history of pertussis in infancy. Many studies have suggested that children with bronchiolitis in infancy caused by viral infections, especially RSV, have a greater risk on developing recurrent wheezing and lower respiratory tract...
infections during early childhood [24-27]. The mechanism behind such an association may be multifactorial. Infections could induce allergic sensitization [25, 28, 29] and/or could affect lung development [30, 31].

Like RSV, pertussis may cause bronchiolitis in young children [32, 33]. Moreover, double infections with *B. pertussis* and RSV or other viruses often occur [34-36]. Possibly, pertussis infection in infancy may cause wheezing by increased bronchial hyper-reactivity either in a similar way as RSV or by co-infection with RSV. Although some of the children in this study had co-infection with RSV, we unfortunately did not inquire this in a standard way. Alternatively, the association may reflect a genetic predisposition for respiratory infections. Previous studies showed that in the majority of children the association between infection in infancy and wheezing in early childhood was transient and related to congenitally smaller airways which predisposed these children to wheezing in association with infection [25, 28, 37, 38]. Conceivably, children with smaller airways may be more prone to severe infection with *B. pertussis* or pertussis is more easily recognized in children predisposed to respiratory morbidity.

Persistent wheezing in children has been shown to be associated with bronchial hyper-reactivity and IgE-mediated sensitization in the first year of life and may be a predictor for asthma in adulthood [23, 28, 39]. We did not measure IgE levels of the children in our study. It might however still be interesting to repeat the measurements in both groups in a few years to determine whether the increased risk on respiratory illness persists in children hospitalised for pertussis in infancy. Although the proportion of children with unsatisfactory scores on language skills in the group with a history of pertussis was higher, we found no statistically significant difference in cognitive development variables between both groups. This may be explained by the absence of children who had neurological complications during their pertussis infection [3]. In particular, hypoxia and brain haemorrhages may affect cognitive functions [5, 12, 40]. Further, the small differences observed may require the inclusion of a larger number of children to attain significant differences.

We acknowledge there are some limitations of the current study. First of all, selection bias may have occurred if participating children in the pertussis group are the ones who suffered most from their pertussis infection in infancy. However, there were no differences in (disease) characteristics between the selected group and the children in the study they were enrolled from [3]. Secondly, information bias may have occurred if parents of children in the pertussis group were more alert on respiratory symptoms since their child suffered from pertussis. The significantly lower weight-for-length in children with a history of pertussis hospitalisation may however be a consequence of an increased risk for respiratory disease. Alternatively, the control group could be overrepresented by children with high respiratory morbidity if their...
parents are more willing to participate in a study on this subject. However, the prevalence of wheezing and respiratory symptoms in the control group was comparable to the prevalence in healthy children of the same age in a large Dutch birth cohort study [41]. It can be argued that an eventual effect on weight or cognitive development is not associated with the pertussis infection itself but with hospitalisation in early infancy. Ideally, to study this, a control group should be included with infants hospitalised for another disease.

The increased circulation of pertussis among adults as observed in many countries poses a significant risk of transmission to unprotected newborns [3, 42, 43]. If there is a causal relation between pertussis in infancy and respiratory morbidity in childhood, this emphasizes even more the need to prevent pertussis in infants. Besides, this will favour estimated cost-effectiveness ratios of vaccination strategies that focus on the prevention of pertussis in infants. More research is required to support the results found in the current study. Future research would ideally be prospective in order to eliminate recall bias, and should focus also on more objective measures (such as lung function or IgE concentrations before and after infection) to elucidate the potential mechanism behind the association. It could be worthwhile to include pertussis diagnostics in studies on the consequences of RSV in infancy and vice versa.

Acknowledgements
We would like to thank all parents and children who participated in the study and all Child Health Centres who provided growth and development records.

References
Pertussis: the figures only represent the 'tip of the iceberg'...

Jökulsárlón, Iceland 2008
Seroprevalence of *Bordetella pertussis* infection during pregnancy measured by IgG antibodies against pertussis toxin

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Abstract

*Bordetella pertussis* infection may cause severe illness in newborns. Mothers with *B. pertussis* infection during delivery can infect newborns. The seroprevalence of *B. pertussis* infection in pregnancy was measured in pregnant women by detection of immunoglobulin G against pertussis toxin; 6.3% had serological evidence of infection. Maternal vaccination should be considered to prevent pertussis in newborns.
Introduction
Whooping cough, caused by *Bordetella pertussis*, may cause severe illness and substantial mortality, especially among neonates [1]. Despite nationwide vaccination programmes with high coverage, the incidence of pertussis is rising in many developed countries during recent decades, with infection frequency assumed to be highest among adolescents and young adults [2]. In the Netherlands, pertussis vaccination is given to infants at the ages of 2, 3, 4, and 11 months. Before 2005, a whole-cell vaccine was used, which was replaced by an acellular vaccine [2]. Since 2001, a booster vaccination with acellular pertussis vaccine at the age of 4 years has been included in the National Immunization Programme. A booster vaccination during adolescence is not recommended.

Infants, who are too young to be (completely) vaccinated, account for the majority of hospital admissions for pertussis [1, 2]. Although adolescents and adults usually are thought to have mild pertussis symptoms, they can be an important source of infection for newborns. Household contacts, especially mothers and siblings, are thought to be responsible for up to 75% of *B. pertussis* infections in infants [3, 4]. Mothers with pertussis at time of delivery have a high chance to infect newborns. Depending on the time of onset of infection of the mother, the neonate may not be protected by maternal antibodies acquired through placental transfer [5].

The aim of this study was to determine the seroprevalence of *B. pertussis* infection in women during and shortly after pregnancy. Knowledge on the infection frequency of *B. pertussis* infection among expectant mothers may support further research to explore the possibilities of vaccination during pregnancy to protect neonates who are still at high risk of severe pertussis.

Methods
All pregnant women who were about to deliver from January 2004 through January 2006 in a general hospital in the Netherlands were asked to participate in the study. After obtaining informed consent, an umbilical cord blood sample was taken and maternal blood samples were taken at delivery and 2 months after delivery. If available, a routinely obtained maternal blood sample at 12 weeks of gestational age was retrospectively analysed.

All samples were frozen at -20°C until analysis. Individual samples were analysed by the National Institute for Public Health and the Environment (RIVM) in the Netherlands. Pertussis specific antibodies against pertussis toxin (IgG-Ptx) were measured with an in-house enzyme-linked immunosorbent assay [6]. A 4-fold increase or decrease of IgG-Ptx to a level of at least 20 U/ml, or IgG-Ptx >100 U/ml in a single sample was indicative of recent *B. pertussis* infection [6]. The IgG-Ptx assay has a lower detection limit of 3 U/ml.
A standardised questionnaire was used to collect information on demographics, vaccination history, coughing complaints, coughing complaints in the household, and general practitioner visits.

This study was approved by the Medical Ethical Committee of the Groene Hart Ziekenhuis. Data were analysed using SPSS, version 12.0.1 (SPSS) and SAS, version 9.1.3 (SAS Institute). Pertussis specific IgG-Ptx levels at different sampling points are reported as geometric mean value with 95% confidence interval (95%CI). To study placental transfer, comparisons of log-transformed levels in maternal delivery serum and in cord serum were tested with a paired Student’s t-test. A P-value <.05 was considered to be statistically significant.

**Results**

During the period of study, 315 mothers were included. The median maternal age at time of delivery was 30 years (range, 15-44 years). The median gestational age was 39.4 weeks (range, 33-43 weeks). Two hundred forty-two women (76%) reported that they were vaccinated against pertussis during childhood, 27 (8.5%) reported that they were unvaccinated, and 46 mothers (15%) had unknown vaccination status.

![Figure 1. Immunoglobulin G antibodies against pertussis toxin (IgG-Ptx) at 12 weeks of gestational age (1), around the time of delivery (2), and 2 months after delivery (3).](image-url)
A distribution of IgG-Ptx levels in the serum of mothers during different sampling times is presented in Figure 1. The majority of women had antibody levels of 3-20 U/ml measured at 12 weeks gestation, at delivery, and at 2 months after birth. Twelve (3.8%) women had antibody levels of 50-100 U/ml in at least one sample. IgG-Ptx concentration below the level of detection (i.e., <3 U/ml) at all measurements occurred in 48 (15.7%) women. IgG-Ptx levels >100 U/ml in at least one sample or a 4-fold increase between 2 consecutive serum samples were detected in 2 (1.8%) of 109 women for whom all samples were available and in 5 (3.4%) of 145 women with 2 samples available. In women with only 1 sample available, 1 (1.6%) of 61 had serological confirmed pertussis. All together, 8 (2.5%) of 315 women had serologically confirmed B. pertussis infection in an 8-month period of follow-up. Therefore, the incidence rate of B. pertussis infection was 3.8 per person-year. Table 1 presents characteristics of women with serologically confirmed B. pertussis infection. Patients 1, 6, and 8 were likely to be infected during pregnancy, because they had very low antibody levels detected in their first sample. Subjects 2, 5, and 7 already showed a high response in their first sample and were likely to have been infected shortly before or in the first weeks of their pregnancy. Only 2 (25%) of these 8 women reported coughing during pregnancy, 1 of whom visited her general practitioner. Pertussis, accompanied with coughing, was noted in both families. Mean IgG-Ptx levels at 12 weeks, at delivery, at 2 months postpartum and in cord serum were 11.9 (95%CI: 9.6-14.2), 10.1 (95%CI: 7.8-12.4), 13.6 (95%CI: 11.3-15.9), and 14.4 (95%CI: 11.8-17.0) U/ml, respectively. The geometric mean level of IgG-Ptx in cord serum in subjects without pertussis was higher than the mean IgG-Ptx levels around the time of delivery (15.7 U/ml [95%CI: 13.5-17.9 U/ml] versus 9.2 U/ml [95%CI: 7.2-11.2 U/ml], P<.01), suggesting some active placental transfer. Active placental transfer is also suggested to occur in subjects with B. pertussis infection (Table 1).

Discussion
Despite high levels of vaccination in childhood, we found serological evidence of B. pertussis infection in 8 (2.5%) of 315 mothers during pregnancy. Another 12 (3.8%) of 315 women had antibody levels of 50-100 U/ml. Although these values do not correspond with active or recent infection, it is indicative of B. pertussis infection in the past year [7]. In total, 20 (6.3%) of 315 pregnant women in our study had serological evidence of B. pertussis infection during or shortly before pregnancy, which is much higher than the incidence of reported cases in this
Table 1. Demographic and clinical characteristics of women with *Bordetella pertussis* infection.

<table>
<thead>
<tr>
<th>Patient</th>
<th>At 12 weeks of gestation</th>
<th>At time of delivery</th>
<th>In umbilical cord</th>
<th>At 2 months after birth</th>
<th>Age</th>
<th>Vaccination</th>
<th>Coughing</th>
<th>Pertussis in family</th>
<th>General practitioner visit</th>
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<td>91</td>
<td>33</td>
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</tr>
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</tr>
</tbody>
</table>
age group [2]. Our findings emphasize once more that *B. pertussis* infection often remains unreported, possibly because of a subclinical course of infection or the failure to recognize or report the disease by a general practitioner.

The incidence we observed is in line with the incidence of 6.6% of *B. pertussis* infection estimated in another study on the seroprevalence of pertussis in the general population in the Netherlands [7]. Likewise, seroprevalence studies in other countries have demonstrated incidences of 3.3%-8% per year in cohorts of adults [8-10]. A recent study conducted in pregnant women [11] found a lower seroprevalence of 1.8%. However, in this study, antibody levels were measured in cord serum. In our study, maternal samples were collected at 3 time points, which increased the diagnostic yield.

Our data suggests efficient placental transfer of maternal IgG-Ptx in pregnant women, which corresponds with findings in other studies [12]. There is evidence that maternal antibodies can offer protection against pertussis in neonates [5]. The majority of women in our study had antibody levels <20 U/ml around the time of delivery (Table 1), and 15.7% had an antibody level below the limit of detection. Although protective levels for antibodies against pertussis have not been established, low IgG-PT levels correlate with increased susceptibility to pertussis [13].

Our study had some limitations. Selection bias may have occurred, because 30% of Dutch pregnant women with uncomplicated pregnancies deliver at home. Women in our study all underwent a hospital delivery. Because obstetrical reasons were responsible for the hospital deliveries we think that our results are still representative. Unfortunately, for logistical reasons, some maternal samples obtained at 2 months after delivery were unavailable. Therefore, we may have underestimated the true number of infections that occurred in mothers shortly after delivery.

The relatively high seroprevalence of *B. pertussis* infection in mothers who are about to deliver illustrates that the bacterium likely circulates within the family and that there is a risk of transmission to the unprotected newborn [14, 15]. Infants can be protected from infection when frequent transmission routes are interrupted by vaccinating all close contacts of newborns, the “cocooning strategy”. In some countries, this strategy has been implemented or recommended. However, the extent to which infant morbidity will be reduced depends on the willingness of contacts to be vaccinated.

A more direct way to protect young infants would be to induce protecting antibodies in these infants by starting to vaccinate directly after birth [16]. However, the type of vaccine and timing will be important for the success of neonatal vaccination; Halasa et al. [17] observed lower antibody responses to acellular pertussis vaccine administered shortly after birth, possibly because of interference [17, 18].
Our results contribute more to the arguments that favour the idea of vaccinating women during the third trimester of pregnancy with an aim to passively protect neonates by elevating maternal antibody levels. The possibilities, advantages, and disadvantages of maternal vaccination have been reviewed elsewhere [5]. Currently, the feasibility of maternal vaccination is hampered by concerns regarding adverse effects of the vaccine in the mother and/or newborn. Thus, further research on safety and effectiveness of maternal vaccination would be very valuable.

References

Seroprevalence of pertussis in the Netherlands: evidence for increased circulation of *Bordetella pertussis*
Abstract

Background: In many countries, the reported pertussis incidence has increased despite high vaccination coverage. However, accurate determination of the burden of disease is hampered by reporting artefacts. The infection frequency is most reliably estimated on the basis of the prevalence of high IgG concentrations against pertussis toxin (IgG-Ptx). We determined whether the increase in reported pertussis in the last decade is associated with an increase in the number of infections.

Methodology/principal findings: In a cross-sectional population-based serosurveillance study conducted in 2006-07, from a randomly selected age-stratified sample of 7,903 persons, serum IgG-Ptx concentrations were analysed using a fluorescent bead-based multiplex immunoassay. In 2006-07, 9.3% (95%CI: 8.5-10.1) of the population above 9 years of age had an IgG-Ptx concentration above 62.5 EU/ml (suggestive for pertussis infection in the past year), which was more than double compared to 1995-96 (4.0%; 95%CI: 3.3-4.7). The reported incidence showed a similar increase as the seroprevalence between both periods.

Conclusions: Although changes in the vaccination programme have reduced pertussis morbidity in childhood, they have not affected the increased infection rate in adolescent and adult pertussis. Indeed, the high circulation of *B. pertussis* in the latter age-categories may limit the effectiveness of paediatric vaccination.
Introduction
In the last decades, an increase of the reported incidence of clinical pertussis cases has been observed in many countries despite high vaccination coverage [1-5]. Various explanations have been given for the pertussis re-emergence, including increased awareness, improved diagnostics, waning of vaccine-induced immunity, and adaptation of the causative pathogen Bordetella pertussis [6-8]. In the Netherlands, despite consistently high vaccination coverage for decades [9], increased numbers of pertussis notifications have been observed since 1996 with epidemic peaks every 2-3 years [1, 2]. A whole-cell pertussis vaccine has been used in the Netherlands since 1953. In 2001, a booster vaccination with acellular vaccine for 4-year-olds was introduced, and in 2005 the whole-cell vaccine was replaced by an acellular vaccine [1]. Previously, we reported that the sudden increase in pertussis notifications in 1996 was largely caused by a genuine increase in clinical pertussis cases and could only partly be explained by changes in diagnostic practice [2]. The increase in reported pertussis cases may be due to a higher circulation of the causative organism B. pertussis and/or to an increase in the fraction of infections which lead to clinical symptoms.

At present, estimations of the true circulation are most reliably made on the basis of the prevalence of high IgG concentrations against pertussis toxin (IgG-Ptx) in the population. In response to an infection with B. pertussis almost all patients show an increase in IgG against pertussis toxin which reaches a maximum within a few weeks. This increase is followed by a steady decline during 6-12 months after infection [10, 11]. Ptx is expressed only by B. pertussis and cross-reacting antigens have not been described. Previously, we showed that an IgG-Ptx level of at least 100 local U/ml (which is equal to 125 EU/ml [12]) is a highly specific criterion for recent pertussis infection [13]. Interpretation of IgG-Ptx concentrations is complicated by the fact that all pertussis vaccines contain Ptx. The Dutch whole-cell vaccine hardly induced IgG-Ptx antibodies. On the contrary, vaccination with acellular pertussis vaccines induces high concentrations of IgG-Ptx, but these rapidly wane within the first 6 months [14-16]. This applies for both primary vaccination in infancy and the booster vaccination at four years of age.

In the current study we aimed to estimate the age-specific seroprevalence of pertussis infections in a cross-sectional sero-survey of the Dutch population in February 2006-July 2007. Based on serodiagnostic cut-off levels of IgG-Ptx we estimated what percentage of the population experienced a recent pertussis infection and what factors are associated with a high IgG-Ptx concentration. To further improve our understanding of the changes in the epidemiology of pertussis over the last decade we compared the age-specific seroprevalence with results obtained from a similar national survey conducted in 1995-1996 [13, 17] and with incidence rates calculated from mandatory notifications in both periods. Our results show that, although
the changes in the vaccination programme have reduced pertussis morbidity in childhood, 
they have not affected the increased infection rate in adolescent and adult pertussis. Indeed, 
the high circulation of \textit{B. pertussis} in the latter age categories may limit the effectiveness of 
paediatric vaccination.

\textbf{Methods}

The study proposal was approved by the Medical Ethics Testing Committee of the foundation 
of therapeutic evaluation of medicines (METC-STEG) in Almere, the Netherlands (clinical 
trial number: ISRCTN 20164309) and all participants provided signed informed consent for 
blood sampling and/or a questionnaire.

\textit{Study population and design}

Between February 2006 and July 2007, a cross-sectional population-based serosurveillance 
study was conducted to estimate for the national population and for different subgroups the age-
specific seroprevalence of antibodies against diseases targeted by the National Immunization 
Programme (NIP) in the Netherlands. Details on study design and data collection were 
described previously [18]. Briefly, from 40 randomly (with probability to their size) selected 
municipalities in the Netherlands an age-stratified selection of individuals (classes 0, 1-4, 
5-9,…75-79 years) was invited to give a blood sample, to fill out a questionnaire and to bring 
their certificates from the National Immunization Programme. To assess the seroprevalence in 
migrants separately, oversampling was performed by inviting a random sample of non-Western 
migrants aged 0-79 years from 12 municipalities included in the nationwide sample. Similarly, 
oversampling was performed in eight municipalities with low immunisation coverage to assess 
the seroprevalence in low vaccination communities (LVCs) and in individuals who refuse 
vaccination based on religious grounds [18]. In total, for 6,385 participants from the national 
sample (including 645 individuals in the oversampled migrant group) and 1,518 individuals 
from areas with low vaccination coverage a serum sample was obtained and a questionnaire 
was completed.

Blood samples were centrifuged (10 min at 2500 rpm) and sera were stored at -80°C until 
analysis. With the questionnaire information was collected on demographic characteristics, 
socio-economic status, educational level, household composition, and vaccination history. 
Furthermore, participants were asked whether and when they had had a period of coughing 
attacks that lasted more than two weeks in the past twelve months, and whether a clinician had 
diagnosed pertussis infection in the past.
Laboratory methods
Serum antibody concentrations were analysed as described previously [19]. In short, total IgG antibodies directed against *B. pertussis*, diphtheria and tetanus (DTaP combo vaccine) were measured simultaneously using a fluorescent bead-based multiplex immuno-assay (DTaP MIA) (Luminex xMAP technology). Analysis was performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA). The MIA showed a high correlation with the FDA ELISA [19] in a large panel of sera with a broad range of concentrations (n=120, Pearson’s correlation coefficient = 0.972, with log(MIA) = 0.9775×log(FDA ELISA)+0.0691). Serum values for *B. pertussis* were assigned in EU/ml [20, 21] as the in-house reference used was calibrated against the U.S. Reference Pertussis Anti-serum Human lot 3 (CBER/ FDA).

Sero-survey 1995-1996
The study design and data collection of the sero-survey conducted in 1995-96 have been published elsewhere [13, 17], and are comparable to the survey in 2006-07. Sera from the national sample (n=7,735) collected from October 1995 until December 1996 were assayed in 1997 in the routine setting of the serology laboratory of the RIVM. IgG-Ptx was measured by enzyme-linked immunosorbent assay (ELISA) as previously described [13, 22]. The IgG-Ptx assay has an upper limit of detection of 500 U/ml and the lower detection limit of the assay is 5 U/ml. Results were expressed in “local” U/ml.

To enable a proper comparison between the two sero-surveys, 217 samples with a broad range of concentrations from persons of all age groups from the 1995-96 survey were retested in the MIA. Concentrations were log-transformed and the Bland–Altman plot demonstrated good agreement between both methods. Due to the use of different references (as reflected by in-house ELISA 100 local U/ml equals 125 FDA ELISA EU/ml [12]) the MIA showed on average 1.28 times higher concentrations (paired t-test with 95%CI: 1.14-1.43). There was a good correlation (with Pearson R=0.890) between the in-house ELISA (X) and the MIA (Y) with log(Y) = 0.0911+1.0118×log(X). This equation was used to transform concentrations measured with the ELISA making them comparable to concentrations obtained with the MIA in the 2006-07 survey. All analyses were further performed with these transformed values.

Statistical analyses
Anti-Ptx IgG levels in both sero-surveys were divided into four categories according to the estimated average time since infection [23]: 0-20 EU/ml, 20 to <62.5 EU/ml, 62.5-125 (infection in the last 6-12 months), and ≥125 EU/ml (infection in the last 6 months). To produce seroprevalence estimates for the Dutch population, each person was assigned a
sampling weight that incorporated the probability of selection and included adjustment for age, gender, urbanisation degree, and ethnicity. Since acellular vaccination may affect the IgG-Ptx immune response in recently vaccinated individuals, results are presented separately for persons targeted with the acellular vaccine (i.e., born after 1998 and ≤9 years of age) and those who were not.

To gain insight into the circulation of pertussis and the level of underreporting, weighted seroprevalence was compared amongst both sero-surveys and with the incidence rates calculated from mandatory notifications. Comparisons are presented as risk ratios (RR) with 95% confidence intervals (95%CI). Details on the mandatory notification system [1, 2] have been described previously.

For the 2006-07 survey, we determined by means of logistic regression analysis, whether there are risk factors for adolescents and adults that are associated with an increased chance on acquiring pertussis infection. Variables with a P-value <0.1 in the univariate model were included in the multivariate model. Backward selection was used to identify covariates that were independently associated with presumptive pertussis infection in the past year. Odds ratios (ORs) for the complete case analysis were calculated and presented with 95% confidence intervals. Analyses were performed using Microsoft Excel and SAS version 9.1.3.

Results

Seroprevalence in children ≤9 years of age

Presumably as a result of the introduction of an acellular booster vaccination for 4-year-olds in 2001 and the replacement of the Dutch whole-cell vaccine by an acellular vaccine in 2005, we observed that children in 2006-07 had generally higher concentrations of IgG-Ptx than children in the 1995-96 survey (Figure 1). In the era of acellular vaccine, 32% (95%CI: 21-43) of the 1-year-old children had an IgG-Ptx concentration ≥62.5 EU/ml, compared to 0.9% (95%CI: 0-2) in 1995-96. However, high levels of IgG-Ptx decreased by age within a year: only 2% (95%CI: 0-5) of the 2-year-olds had an antibody concentration ≥62.5 EU/ml in 2006-07, while 91% (95%CI: 86-96) had a concentration below 20 EU/ml. Due to the booster, 11% (95%CI: 6-16) of the 4-year-olds had an IgG-Ptx concentration ≥62.5 EU/ml and this decreased with age to 4% (95%CI: 0-7) in 7-year-olds. In 8- and 9-year-olds the fraction with a concentration ≥62.5 EU/ml was increased again to 9% (95%CI: 3-15) and 10% (95%CI: 4-16), respectively.
Figure 1. Age-specific seroprevalence of IgG-Ptx concentrations in children aged 0-9 years in 1995-96 (upper figure) and in 2006-07 (lower figure). Note: on the x-axis the age-group, number tested and in brackets the percentage targeted by the acellular vaccine are indicated. In 2006-07, children aged 4 years could have been primed with either whole-cell or acellular vaccine in infancy (nationwide coverage circa 96%), and children aged 4-9 years could have been primed with whole-cell vaccine and may have received a preschool booster with acellular vaccine (nationwide coverage circa 90%).
Seroprevalence in persons >9 years of age

Figure 2 shows the age-specific seroprevalence based on IgG-Ptx in the national sample in 1995-96 and in 2006-07, in adolescents and adults aged >9 years. Overall, 4.0% (95%CI: 3.3-4.7) of the people aged >9 years in 1995-96 had an IgG-Ptx concentration ≥62.5 EU/ml (presumptive pertussis infection in the past twelve months) and 1.0% (95%CI: 0.7-1.2) ≥125 EU/ml. In 2006-07, these percentages were 9.3% (95%CI: 8.5-10.1) and 3.4% (95%CI: 2.8-3.9), respectively. Overall, the seroprevalence of concentrations ≥125 EU/ml (presumptive pertussis infection in the past six months) was in 1995-96 a factor 116 and in 2006-07 a factor 100 higher than the reported incidence at the time. Figure 3 shows the age-specific risk ratios for the comparison of seroprevalence and reported incidence between the two serosurveys. Per age group the reported incidence was 3 to 7 times higher in 2006-07 than in 1995-96. Interestingly, the prevalence of individuals with an antibody concentration ≥125 EU/ml showed similar increases, except for the 20-34 and 50-64 years age group where this increase was less pronounced.

The prevalence of reported coughing among people with an IgG-Ptx concentration ≥62.5 EU/ml increased from 17% (95%CI: 11-24) in 1995-96 to 25% (95%CI: 20-29) in 2006-07 (Table 1). Especially among the 35-49 and 65-79 years age group an increase was seen. In the 2006-07 survey 19 persons (0.4%) reported to be diagnosed with pertussis in the past year, 8 of them had an IgG-Ptx concentration ≥62.5 EU/ml. In 1995-96, 8 (0.1%) persons were diagnosed with pertussis in the past year; none had an IgG-Ptx concentration ≥62.5 EU/ml.

With the multivariate regression model including gender, age, income and religion, it was found that male compared to female, people aged 65-79 years compared to adolescents, people with low incomes compared to high income groups, and people from Reformed Congregations compared to people without religious background, were more likely to have an IgG-Ptx concentration ≥62.5 EU/ml (Table 2).

### Table 1. Prevalence (%) of coughing symptoms in the preceding year, in individuals with IgG-Ptx concentration ≥62.5 EU/ml, by age group, and period (1995-96 versus 2006-07).

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<tr>
<th>Age group</th>
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</table>
Figure 2. Age-specific seroprevalence of IgG-Ptx concentrations in individuals aged >9 years in 1995-96 (upper figure) and in 2006-07 (lower figure).
Figure 3. Risk ratios and 95% confidence intervals for the comparison of seroprevalence and reported incidence of pertussis in individuals aged >9 years in 2006-07 versus 1995-96.
Table 2. Risk factors for an IgG-Ptx concentration >62.5 EU/ml in individuals aged >9 years in 2006-07 (n=5830)×.

<table>
<thead>
<tr>
<th>Category</th>
<th>No (%) with IgG-Ptx concentration &gt;62.5 EU/ml</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>287 (11)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Female</td>
<td>293 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-19 years</td>
<td>81 (9)</td>
<td>0.8 (0.7-0.9)</td>
<td>0.8 (0.7-0.9)</td>
</tr>
<tr>
<td>20-34 years</td>
<td>128 (10)</td>
<td>1.1 (0.8-1.4)</td>
<td>1.1 (0.8-1.5)</td>
</tr>
<tr>
<td>35-49 years</td>
<td>109 (9)</td>
<td>1.0 (0.7-1.3)</td>
<td>1.0 (0.8-1.4)</td>
</tr>
<tr>
<td>50-64 years</td>
<td>118 (9)</td>
<td>1.0 (0.7-1.3)</td>
<td>1.0 (0.8-1.4)</td>
</tr>
<tr>
<td>65-79 years</td>
<td>144 (12)</td>
<td>1.4 (1.0-1.8)</td>
<td>1.4 (1.0-1.9)</td>
</tr>
<tr>
<td>Ever vaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>139 (11)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>190 (9)</td>
<td>0.8 (0.7-1.0)</td>
<td></td>
</tr>
<tr>
<td>Living in urban area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>227 (11)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>353 (9)</td>
<td>1.2 (1.0-1.4)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch or western migrant</td>
<td>531 (10)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Turkish + Moroccan</td>
<td>16 (12)</td>
<td>1.3 (0.7-2.1)</td>
<td></td>
</tr>
<tr>
<td>Other non-western migrant</td>
<td>33 (9)</td>
<td>0.9 (0.6-1.4)</td>
<td></td>
</tr>
<tr>
<td>Persons in household</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>286 (10)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>203 (10)</td>
<td>1.0 (0.8-1.2)</td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>91 (10)</td>
<td>1.0 (0.8-1.2)</td>
<td></td>
</tr>
<tr>
<td>Household member ≤4 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>73 (10)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>507 (10)</td>
<td>1.0 (0.8-1.3)</td>
<td></td>
</tr>
<tr>
<td>Number of rooms in the house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>32 (11)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>3-6</td>
<td>425 (10)</td>
<td>0.9 (0.6-1.3)</td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>123 (9)</td>
<td>0.8 (0.5-1.2)</td>
<td></td>
</tr>
<tr>
<td>Net monthly income per household</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ € 3,050</td>
<td>386 (10)</td>
<td>1.5 (1.2-2.0)</td>
<td>1.5 (1.1-1.9)</td>
</tr>
<tr>
<td>&gt; € 3,050</td>
<td>64 (7)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Unknown</td>
<td>130 (10)</td>
<td>1.5 (1.2-2.1)</td>
<td>1.5 (1.1-2.1)</td>
</tr>
<tr>
<td>Educational levelb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>81 (11)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Middle</td>
<td>308 (10)</td>
<td>0.9 (0.7-1.1)</td>
<td></td>
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<tr>
<td>High</td>
<td>191 (10)</td>
<td>0.8 (0.6-1.1)</td>
<td></td>
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<tr>
<td>Religion</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No religion</td>
<td>188 (10)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Reformed Congregations</td>
<td>75 (13)</td>
<td>1.4 (1.0-1.8)</td>
<td>1.3 (1.0-1.7)</td>
</tr>
<tr>
<td>Other</td>
<td>317 (10)</td>
<td>1.0 (0.8-1.2)</td>
<td>0.9 (0.8-1.1)</td>
</tr>
</tbody>
</table>

× For 179 persons (3%) data on one or more variables was missing.

b In children aged <14 years the mothers highest educational level was asked; low = no education or primary education; middle= junior technical school, lower general or intermediate vocational secondary education; high= higher vocational or higher general secondary education, pre-university or university education.
Discussion

In this national randomized sero-survey we found that in 2006-07 approximately 9% of the population in the Netherlands aged >9 years had a pertussis infection in the preceding year. This percentage has more than doubled compared to 1995-96. The increased seroprevalence is consistent with the steady increase in reported and hospitalised cases in adolescents and adults in the past decade [1], illustrating that the observed increase in pertussis is not only due to improved reporting rate or better awareness but rather to a real increase in the circulation of pertussis. About a quarter of the adolescents and adults with presumptive pertussis infection reported to have had at least two weeks of coughing symptoms in the preceding year. These findings support the concept of endemic, though often mitigated, pertussis among adolescents and adults, in a country with a high coverage childhood vaccination programme.

Increased levels of IgG-Ptx induced through vaccination with acellular pertussis vaccines cannot be distinguished from high levels due to infection, and the high seroprevalence in children below 8 years of age in the 2006-07 survey is likely to be induced by vaccination and not by natural infection [15, 24]. However, the increase with age from 7 years onwards in the proportion of children with an IgG-Ptx concentration ≥62.5 EU/ml suggests natural infection may occur again two years after the preschool booster. As the Dutch whole-cell vaccine hardly induces IgG-Ptx antibodies [16], high levels measured in the group of participants older than 9 years and not eligible for the acellular vaccine are most likely due to recent infection. The seroprevalence of presumptive infections deduced from the 1995-96 survey was similar to that reported in other countries with high vaccination coverage [25, 26]. Compared to 1995-96, the seroprevalence in 2006-07 had increased with a factor of almost 3 in adolescents and adults, and up to 8 in elderly. Similar increases were observed among adults aged 35-59 years in Australia in a comparison of two serological studies performed in 1998 and 2002 [27]. In Sweden, re-introduction of pertussis vaccination in childhood decreased the circulation in children, however in Swedish adolescents aged 14-18 years and in elderly aged >65 years the proportion of persons with an IgG-Ptx concentration indicative for recent infection became higher, suggesting an increased circulation of B. pertussis in older age categories [28].

We propose that the increase in the circulation of B. pertussis can be attributed to a combination of waning immunity and the emergence of new strains [7, 8, 29]. In the Netherlands, the use of an imperfect whole-cell vaccine in the beginning of the nineties may have increased the level of circulation in the population [30]. Concurrently, a change in circulating B. pertussis strains was observed [31]. Recently, it was shown that the strains circulating since the end of the 1990s contain a mutation which confers increased pertussis toxin which may enhance infection of primed hosts [29]. There is also evidence that these so-called P3-strains are more virulent. Consistent with this, we observed that the increase in reported cases was more pronounced
than the increase in infections and a larger part of the infections resulted in clinical symptoms in 2006-07. The upward trend by age of the proportion of presumptive pertussis in adults may indicate that the more immunity has waned the higher the chance to become infected by *B. pertussis*. In children, however, the introduction of a preschool booster and the replacement of the Dutch whole-cell vaccine by an acellular vaccine, led to improved protection of the cohorts born after 1998 [1].

The higher seroprevalence in low income groups may be related to lower hygiene measures. Interestingly, people from Reformed Congregations - who are known to refuse vaccination for religious reasons - had higher rates of infection, possibly as a result of the absence of vaccine-induced immunity.

We acknowledge there are some limitations of the current study. First of all, a major concern could be the use of different laboratory techniques to measure IgG-Ptx levels. The 1995-96 samples were tested with the RIVM ELISA, while the 2006-07 sera were tested in a multiplex immuno-assay (MIA). However, accounting for use of different references, the results obtained with the in-house ELISA in 1995-96 turned out to be similar when tested with the MIA. Noticeably, for international comparison, the values generated with the MIA showed a high correlation with the FDA ELISA [19]. Secondly, it should be noted that this is not a longitudinal study with two time points but a comparison between two cross-sectional studies. Considering the periodicity in pertussis with peaks every 2-3 years [32], the incidence and seroprevalence in 2006-07 may be at the high end of the normal range burden in the past decade. However, based on the similarity between the increase in the prevalence of a concentration ≥125 U/ml and the reported incidence, it may be assumed that both gradually increased during the years in between the two surveys. Finally, from April 1997 a single serum sample above the diagnostic cut-off was included in the case definition for notification [2]. This means that from that time a higher proportion of patients could be notified which may partly explain the increase in reported pertussis, though not in seroprevalence.

Our results show that, although the improvements in the vaccination programme have reduced pertussis morbidity in childhood, they have not affected the increase in adolescent and adult pertussis. The high circulation rates of *B. pertussis* in the latter age categories may have even limited the effectiveness of paediatric vaccination. High circulation of *B. pertussis* among adults - which is the compound effect of waning immunity and pathogen adaptation - may support the idea of introducing adult booster vaccinations [33]. However, investing in an expensive and labour-intensive recurring vaccination programme for adults to boost their immunity seems paradoxical when under the current situation natural boosting takes place frequently and results in relatively mild infections in adults. When transmission remains high, mild infections among adults will be frequent, boosting clinical immunity and hence low levels
of severe disease may be maintained [34]. We therefore believe it would be more efficient to invest in the development of improved vaccines which induce long lasting immunity to reduce the pertussis disease burden. For the short term however, the high circulation emphasizes the need for good protection of unvaccinated infants who are at highest risk for severe pertussis. Vaccinating people in close contact with infants, will be an (cost)effective strategy to prevent transmission of pertussis to infants [35].

References


SEROPREVALENCE IN GENERAL POPULATION

6
Parents and siblings play an important role in transmission of pertussis to infants. This finding was confirmed by the results of a household transmission study named BINKI (which is an acronym in Dutch for: Baby’s geINfecteerd met KInkhoest).
Seasonal patterns in time series of pertussis

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Arnold L.M. Dekkers
Peter Teunis
Janette C. Rahamat-Langendoen
Frits R. Mooi
Hester E. de Melker

Epidemiolog Infect 2009;137:1388-1395
Abstract
To gain insight into pertussis disease dynamics, we studied age-specific long-term periodicity and seasonality of pertussis in the Netherlands. Hierarchical time-series models were used to analyse the monthly reported pertussis incidence in January 1996 – June 2006 by age group. The incidence of pertussis showed a slightly increasing long-term trend with highest incidence rates seen in 1996, 1999, 2001, and 2004. For all age groups the annual peak incidence was found in August, except for 13-18 years age group where the peak occurred in November. Monthly trends in adults showed high correlation with trends in 0-4 years (0.94) and 5-12 (0.92) year olds. We found no evidence for a relationship between annual rises in pertussis and the opening of schools. Concurrent annual fluctuations of pertussis incidence in adults and infants suggest frequent transmission within and between these age groups. Studying trends offers insight into transmission dynamics and may facilitate decisions on future vaccination strategies.
Introduction
Pertussis, or whooping cough, is a highly infectious respiratory disease mainly caused by Bordetella pertussis and more rarely by Bordetella parapertussis. Unvaccinated infants are at greatest risk for severe complications or death due to pertussis. At the beginning of the twentieth century, before vaccines were widely used, epidemics of pertussis were observed to recur at intervals of 2-3 years [1]. Since the introduction of vaccination in the 1950s, the incidence of pertussis has strongly decreased. However, even in countries with high vaccination coverage, pertussis shows epidemic peaks every 3-4 years [2-4]. Moreover, some studies report pertussis has seasonality which is not consistent in time and place [3, 5, 6], although other studies have shown seasonality to be absent during periods of high vaccine uptake [7]. Seasonal increases are a common phenomenon in infectious diseases, but underlying mechanisms are not completely clear [8]. For respiratory pathogens seasonal increases are thought to be driven by seasonal variations in survival of the pathogen outside the host, host behaviour, and the level of host immunity [9]. Opening of schools with subsequent crowding of susceptible persons has been described as one of the major contributors to the annual rise in measles [10] and may also play a role in seasonal increases in the incidence of pertussis [3, 11, 12]. In the last decades an upsurge in incidence has been observed in many industrialized countries with a continuously high vaccination coverage, especially in adolescents and adults [13-15]. Consequently, new vaccination strategies for pertussis are currently under discussion in many countries [16]. Studying seasonal trends for pertussis may reveal important routes of transmission and help to eventually give insight into the possible impact of future vaccination strategies. We analysed long-term trends and age-specific differences in seasonality for pertussis in the Netherlands during the years 1996-2006, and scrutinized the effect of school opening on the incidence of pertussis.

Methods
Disease surveillance
Pertussis is a statutory notifiable disease in the Netherlands. Since 1988, the case definition for notification includes a clinical presentation compatible with pertussis (i.e., serious cough with a duration of >2 weeks and/or coughing attacks and/or cough followed by vomiting) in combination with: isolation of B. pertussis or B. parapertussis, detection of B. pertussis or B. parapertussis DNA by PCR, or a significant rise in IgG antibodies against pertussis toxin or IgA antibodies against whole-cell sonicate of B. pertussis in paired serum samples, or a single serum sample with IgA/IgG-titres above a defined age-specific cut-off value [17], or contact
in the last 3 weeks with a laboratory-confirmed patient with \textit{B. pertussis} or \textit{B. parapertussis} infection. Notifications for pertussis during the years 1989-2006 were collected by week and month of onset and stratified by age groups: 0-4 years (children at home); 5-12 years (children attending primary school); 13-18 years (adolescents attending secondary school); and 19-99 years (adults).

\textbf{Statistical analysis}

We aimed to model the expected monthly incidence for each age group, corrected for long-term trends, autocorrelation and monthly trends. We allowed $Y_t$, $t=1, 2, ..., 126$ to denote the reported pertussis incidences for month $t$ from January 1996 until June 2006. Since the time series $Y_t$ are expected to show autocorrelation, Poisson models with autocorrelated errors are required to correctly describe the time series of the pertussis incidences [18-20]. We used hierarchical time-series models using a procedure called Non-Parametric empirical Bayesian Time Series Analysis (NPBats) [18, 21]. The basic idea is to assume simple prior models for modelling the difference between two or more consecutive observations in a time series. In contrast to ARIMA models for time series - where all observations are used to calculate the expected value at time $t$ and the relationship between two consecutive observations is fixed - the autocorrelation between observations in this method is modelled locally with a moving window of variable width.

We allowed $\mu_t = E(Y_t)$ to denote the expected incidence of reported pertussis cases in month $t$. We defined a generalized linear model [22] with log link function. The expected incidence for month $t$ is then given by

\[
\mu_t = e^{\eta_t}, \quad t = 1, 2, ..., N
\]

and

\[
Y_t \mid \mu_t \sim \text{Poisson} (\mu_t), \quad t = 1, 2, ..., N
\]

Equation (1) relates the expected pertussis incidences, $\mu_t$ for month $t$ to the unknown parameters $\eta_t$, which are of interest since they can be expressed as

\[
\eta_t = X_t \beta_t
\]

where $X_t$, ($t=1, 2, ..., 126$) (10.5 years of months) denote the co-variables and $\beta_t = (\beta_{0t}, \beta_{1t}, ..., \beta_p)^T$ the unknown regression coefficients, with $p$ the number of covariates, e.g., $p=12$ when time
is included as a continuous covariate and 11 dummy variables are used to model the specific contribution of each month compared to January. Note that all covariates are assumed constant over time and that $\beta_0$ depends on the time of sampling, thus accounting for the autocorrelation in the time series $Y_t$.

As in Heisterkamp et al. [18] we propose four nested models for the intercept $\beta_0$, ranging from no autocorrelation to autocorrelation comparable with a second-order autoregressive model.

The four models for the time-dependent, random intercept of equation (3) are

\begin{align*}
\beta_0 | \beta_{0,-1}, \ldots, \beta_{0,t-1} &\sim \text{Normal}\left(\beta_0, \lambda^{-1}\right) \\
\beta_0 | \beta_{0,-1}, \ldots, \beta_{0,t-1} &\sim \text{Normal}\left(\beta_{0,t-1}, \lambda^{-1}\right) \\
\beta_0 | \beta_{0,-1}, \ldots, \beta_{0,t-1} &\sim \text{Normal}\left(\beta_{0,t-1} + (\beta_{0,t-1} - \beta_{0,t-2}), \lambda^{-1}\right) \\
\beta_0 | \beta_{0,-1}, \ldots, \beta_{0,t-1} &\sim \text{Normal}\left(\beta_{0,t-1} + (2\beta_{0,t-1} - 3\beta_{0,t-2} + \beta_{0,t-3}), \lambda^{-1}\right)
\end{align*}

Model (4) is designated the mean prior model: a stationary intercept $\beta_0$ and a normally distributed noise with variance $\lambda^{-1}$. Model (5) is designated a neighbour model: the expectation at time $t$ is assumed equal to its value at time $t-1$. In other words, the difference of two consecutive $\beta_0$'s is normally distributed with mean zero and variance $\lambda^{-1}$. Model (6) is designated linear since the expectation of the difference of two consecutive differences is zero and finally model (7) is designated quadratic since it consists of the second-order difference of three consecutive differences.

All four models are applied to the time series and the one with the lowest Akaike Bayesian Information Criterion (ABIC) was selected as the best one [18, 21]. The estimated month effects are reported as rate ratios in respect of January. Pearson correlation coefficients were calculated to compare month effects between age groups. Furthermore, the best-fit model per age group was used to predict monthly incidences for July 2006 to June 2007, which were compared with observed data for the same period.

To assess the possible relationship between seasonality of pertussis and the crowding of children at schools after holiday periods, the week effects in the incidence of notified cases in 5-12 and 13-18 years age groups during 2000-2004 were assessed using the same models as described above, but now with week as covariate. Modelled peak incidences were set against date of school opening after summer holidays. Because of the staggering of summer holidays regimes in the Netherlands, opening of schools is in weeks 32, 33 or 34.

Data analyses were performed with SAS 9.1, Excel, and S-PLUS 6.2. A P-value <.05 was considered statistically significant.
**Results**

The highest incidence of notified pertussis cases per 100,000 were seen in 1996, 1999, 2001, and 2004 for all groups. This suggests a periodic behaviour, but not with a fixed period. The annual mean of the incidence varied from 4.1 to 20.5/100,000 for the 0-4 years age group and from 0.3 to 2.2/100,000 for adults (Figure 1). The figures suggest an increasing linear long-term trend in incidence over the years for 13-18 years group and adults. Including time as a covariable into the models showed that these increasing trends were not statistically significant.

**Figure 1.** Monthly reported incidence rates per 100,000 (open circles) and best-fit model for the period January 1996 - June 2006 in each age group. The lines represent the predicted incidence per 100,000. The shaded areas represent the 95% confidence bounds for the predicted incidence.
The estimated coefficients of the model with the lowest ABIC per age group are given in Figure 2. The neighbour model was the best-fit model for age group 0-4 years, the linear model for age groups 5-12 and 13-18 years, and the quadratic model for the adult group. Month effect was significant in all models.

Figure 2 shows that August is the month with the highest month effect for age groups 0-4, 5-12 years and adults, i.e., the predicted incidence in these groups increases compared to January with a factor 2.86 (95% confidence interval (95%CI): 2.30-3.55), 1.48 (95%CI: 1.19-1.83) and 1.97 (95%CI: 1.47-2.65), respectively. For the 13-18 years age group the peak is in November, when the predicted incidence increases with a factor of 1.36 (95%CI: 1.12-1.66) compared to January.

In Table 1 the correlations of monthly effects between age groups are given. Trends in adults show a high correlation with trends in 0-4 (0.94) and 5-12 years (0.92) age groups.

**Table 1.** Correlation coefficients between the month effects (corrected for autocorrelation) on reported pertussis incidence per 100,000 by age group in the period January 1996-June 2006.

<table>
<thead>
<tr>
<th></th>
<th>Age 0-4</th>
<th>Age 5-12</th>
<th>Age 13-18</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 0-4</td>
<td>1.00</td>
<td>0.88</td>
<td>0.36</td>
<td>0.94</td>
</tr>
<tr>
<td>Age 5-12</td>
<td>0.88</td>
<td>1.00</td>
<td>0.69</td>
<td>0.92</td>
</tr>
<tr>
<td>Age 13-18</td>
<td>0.36</td>
<td>0.69</td>
<td>1.00</td>
<td>0.52</td>
</tr>
<tr>
<td>Adults</td>
<td>0.94</td>
<td>0.92</td>
<td>0.52</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 3 shows the forecasted monthly incidence (solid curve) and the observed incidence of notifications (black symbols) for July 2006 to June 2007. Forecasts are given with a corresponding 97.5% lower bound. For the 0-4 years age group the forecasted incidence largely corresponds to the observed incidence. In the older age groups the observed incidence exceeds the estimated incidence in the first half of 2007, confirming our observations from routine surveillance data that 2007 is again an epidemic year.

Figure 4 shows the estimated week effects by age group for the period 2000-2004 in relation to the opening of schools. For primary school children, aged 5-12 years, the incidence peaks in week 5, 18 and 31, while in children attending secondary school peaks are observed in week 18, 27 and 31.

Figure 3. Monthly observed incidence of reported cases (black dots) and forecasted incidence (black line) per 100,000 based on the best-fit model per age group for January 1996 to June 2006. The dotted lines represent the 97.5% lower bound for the forecasted predicted incidence. The upper bound is not shown since it varies from 0.06 to ~3500 cases/100,000.
Discussion

Despite a nationwide vaccination programme with high coverage, every 2-3 years a peak in the incidence of pertussis has been observed in the Netherlands in the last decade. Moreover, a clear seasonal pattern in the incidence of reported cases is seen, with most cases occurring in the third quarter of the year. For all age groups pertussis is most frequent in August, except for the 13-18 years group where the peak occurs in November. We found no evidence for a relationship between the annual increase in pertussis and the opening of schools. However, high correlations between the monthly trends in children aged 0-4 and 5-12 years, and adults suggest frequent transmission of pertussis within families.

Periodic epidemics of pertussis have also been reported for other countries [2]. Such oscillations in the occurrence of a disease in highly vaccinated populations are poorly understood. In case of pertussis, the epidemiology and transmission dynamics are driven by the interplay between
waning immunity and boosting by infection or vaccination [4, 23, 24]. In addition, changes in the B. pertussis population may contribute to trends in the epidemiology of pertussis [25, 26]. Although previous results have been conflicting, some studies have suggested that high levels of immunization increase the inter-epidemic period for pertussis by reducing the effective reproduction number [3, 7, 27]. Grenfell and Anderson [28] demonstrated that the expected effect of vaccination on the increase of the inter-epidemic period fails to occur when vaccine efficacy is suboptimal or when there is rapid waning of immunity. Thus, the relative short inter-epidemic period we found in the highly vaccinated population of the Netherlands in the last decade may be explained by a low effective immunization coverage due to reduced quality and duration of vaccine-induced immunity of the Dutch vaccine in the mid-1990s [29]. In addition, the increase in the number of infections among adolescents and adults does indeed indicate a transient increase in the number of susceptible individuals, probably as result of waning of vaccine-induced immunity in combination with the emergence of new strains [30]. We acknowledge that the introduction of PCR as diagnostic method may have contributed also to the observed increase of pertussis in adults, since a larger proportion of infections can now be diagnosed. However, as only about 5% of reported cases are confirmed by PCR in the Netherlands, this effect will only be marginal [24].

Besides epidemic peaks, we found a clear seasonal pattern in the occurrence of pertussis cases. It may be argued that seasonality in pertussis notification data is caused by variations in reporting rate, i.e., a lower reporting rate in winter as other respiratory pathogens are considered in the differential diagnosis. However, the fact that the proportion of positive serological tests for pertussis increases in the same months (data not shown), indicates that there is a true increase of pertussis patients in these months. We acknowledge that notification data considerably underestimate the true incidence of infections as there will be many more ‘subclinical’ cases [31]. Apparently, variation in host- or pathogen-related factors are important in accounting for seasonal changes in pertussis. Climate or environmental factors may indeed affect susceptibility or survival of the pathogen outside the host [9].

Regarding measles [10], some authors have suggested there might be an association between opening of schools and an annual increase in pertussis incidence [3, 7, 28]. School outbreaks of pertussis demonstrate that schoolmates play an important role in the transmission of pertussis [11, 32]. Our data do not support a strong relation between opening of schools after summer holiday and the start of the annual rise in incidence of pertussis. First, if crowding of individuals after school openings was the driving force for the annual rise, one would expect a peak shortly after opening of schools in week 32-34. Here, we see peaks occurring almost every 4 weeks. The latter may reflect that the exact date of onset is often set at the first day of the month if only the month of disease onset is known. Second, in August, the seasonal
increase in preschool-children and adults is much more pronounced than in schoolchildren. Interestingly, in a Canadian study a seasonal increase of pertussis in schoolchildren was found in June during the academic year, while in infants and preschool-children the typical peak months August and September were maintained [5]. These findings might suggest schoolchildren become infected after an exhaustive school year and spread the infection to their family members during summer holidays.

We can think of two reasons why the effect of school opening is not observed for pertussis as for measles. First, because of waning immunity for pertussis not all infected persons have typical symptoms and are diagnosed as pertussis cases [31]. However, subclinical infections can still contribute to transmission. In relation to this, the transmission of pertussis appears to be very efficient within households, resulting in high attack rates even among vaccinated persons [33]. Second, the incubation period for pertussis is highly variable and the infectious period is longer than for measles. Subclinical infections and heterogeneity in infectious period may impede any observable effect of school opening on pertussis incidence.

Following the persistently high incidence of pertussis in 1996 - 2001 an acellular preschool booster has been introduced in the Dutch vaccination schedule. After introduction of this preschool booster the incidence in the targeted age groups declined and some herd immunity effect among infants was observed [24]. Correspondingly, the high correlation between the seasonal distribution of reported cases in children aged ≤4 and 5-12 years probably reflects frequent transmission of pertussis between those groups. Like Tanaka [6], we found a high correlation between annual trends in reported cases in young children and adults, while there was a low correlation between teenagers and other groups. Interestingly, these findings are in line with results from totally different types of epidemiological studies. Several household studies have pointed at the prominent role of parents in the transmission of pertussis to children [34, 35]. In a population-based prospective survey (POLYMOD), Mossong et al. [36] showed that adolescents mixed preferentially with people of the same age, while young children also mixed with middle-aged adults.

Although annual trends were quite similar in age groups, the selected (best-fit) models to describe these trends differed, indicating different degrees of autocorrelation within each age group: the neighbour model for children, the linear model for the 5-18 years group and the quadratic model for adults. From an epidemiological viewpoint one might speculate that this indicates that there are differences in infectious periods between these groups. The quadratic model for adults may suggest that adults have a relatively long infectious period compared to the younger age groups, corresponding to the fact that in adults transmission might continue during a longer period as the disease is often not immediately recognized.
Even though the mechanisms responsible for seasonal changes are poorly understood, studying the variation in seasonal trends provides insight into transmission dynamics of pertussis. Paralleling annual trends in incidence in adults and young children, suggests frequent transmission within families. As long as vaccines do not offer lifelong protection, this finding supports the introduction of vaccination strategies such as cocooning, that aim to discontinue the most frequent paths of transmission to those that are at highest risk for severe infection, i.e., infants.

Acknowledgements
We thank the staff of the municipal public health services concerned with infectious diseases in the Netherlands for collecting and documenting the data requested in the notification system.

References
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Pertussis disease burden in the household: how to protect young infants

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Abstract

Background: We conducted a population-based, nationwide, prospective study to identify who introduced pertussis into the household of infants aged <6 months admitted to the hospital for pertussis in the Netherlands.

Methods: During the period 2006-2008, a total of 560 household contacts of 164 hospitalised infants were tested by polymerase chain reaction, culture, and serological examination to establish *Bordetella pertussis* infection. Clinical symptoms and vaccination history were obtained by a questionnaire submitted during sample collection and 4-6 weeks afterwards.

Results: Overall, 299 (53%) household contacts had laboratory-confirmed pertussis; 159 (53%) had symptoms compatible with typical pertussis infection, and 42 (14%) had no symptoms. Among children vaccinated with a whole-cell vaccine, 17 (46%) of 37 had typical pertussis 1-3 years after completion of the primary series, compared with 9 (29%) of 31 children who had been completely vaccinated with an acellular vaccine. For 96 households (60%), the most likely source of infection of the infant was established, being a sibling (41%), mother (38%) or father (17%).

Conclusions: If immunity to pertussis in parents is maintained or boosted, 35-55% of infant cases could be prevented. Furthermore, we found that, 1-3 years after vaccination with whole-cell or acellular vaccine, a significant percentage of children is again susceptible for typical pertussis. In the long term, pertussis vaccines and vaccination strategies should be improved to provide longer protection and prevent transmission.
Introduction
Vaccination has strikingly reduced the morbidity and mortality due to pertussis in developed countries [1]. However, in the past decade, a resurgence of pertussis has been experienced in many countries that has been attributed to increased awareness, waning immunity, and pathogen adaptation [2-6]. The high circulation of pertussis in vaccinated populations puts 0-6 month-old infants at risk, because they are too young to be completely vaccinated. Pertussis-related morbidity and mortality are at its highest in this group.

To protect infants against pertussis, the main sources of their infection must be identified. Several studies have demonstrated high attack rates for pertussis after household exposure, showing that adults played an important role in the transmission to children [7-13]. However, the contribution of various household members cannot be based on these studies, because they were designed for other aims. Moreover, they identified cases largely on clinical diagnosis, because laboratory confirmation was suboptimal or not available for all household members.

Two multinational studies involving only small numbers of subjects recently attempted to clarify the sources of pertussis infection in young infants [14, 15] and concluded that parents, especially mothers, were the source in most cases. They suggested that vaccination of parents could substantially reduce the burden of infant pertussis.

We conducted a population-based, nationwide, prospective study to identify which household members had introduced pertussis to an infant hospitalised for pertussis. Both clinical and laboratory (culture, serological testing, and polymerase chain reaction (PCR)) criteria were used to maximize diagnostic yield and to identify infected persons more reliably. Furthermore, we assessed the age-specific attack rate, severity, and impact of pertussis in a household setting and determined the effectiveness of vaccination in high-exposure settings.

Methods
Population
From 1 February 2006 through 30 November 2008, pediatricians, microbiologists, and local public health services in the Netherlands reported any infant aged <6 months hospitalised with *Bordetella pertussis* or *Bordetella parapertussis* infection to the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment (RIVM). Upon agreement of the parents or caretakers of the infant (hereafter denominated as ‘index infant’), a home visit was promptly conducted by the study nurse. Information on demographic characteristics, pertussis vaccination status, symptoms, hospital stay, family composition, and coughing contacts of the infant was collected by interviewing parents using a standardized questionnaire. Furthermore, household contacts (i.e., persons living in the same house as the
index infant) were enrolled in the study after providing informed consent and were interviewed using a standard questionnaire on demographic characteristics, vaccination history, pertussis history, clinical symptoms in the past 2 months, coughing contacts, and work loss. To identify *B. pertussis* or *B. parapertussis* by PCR or culture, nasopharyngeal and buccal swab specimens were collected from all household contacts, and a blood sample was obtained for pertussis serological testing. Four to 6 weeks after the initial home visit, follow-up data on symptoms were collected by phone for all participants.

Vaccination status of infants and children aged <13 years was obtained via the national register. During the study period, pertussis vaccination was offered within the National Immunization Programme (NIP) at 2, 3, 4, and 11 months and – since 2001 – an acellular preschool booster was offered at 4 years of age. Since 2005, an acellular vaccine has replaced the Dutch whole-cell vaccine for vaccinations in infancy. Ethics approval for our study was obtained from the Medical Ethical Committee of the University Medical Centre of Utrecht.

**Diagnostic laboratory procedures**

Laboratory confirmation was based on culture, PCR, or serological test results. A nasopharyngeal swab was used to inoculate Regan-Lowe charcoal agar containing 40 μg/mL cephalaxin. The Regan-Lowe plates were incubated at 35°C in high humidity and were examined daily for 14 days for colonies typical of Bordetella species. Colonies were confirmed by partial sequencing of the Bordetella pertactin and toxin gene [16]. Specimens for PCR were collected as a nasopharyngeal or buccal swab (Dacron). PCR tests were performed in the regional public health laboratory in Tilburg, the Netherlands. Swabs were rinsed in 1 mL of solution containing 150 mmol/L NaCl and 1 mmol/L EDTA. Nucleic acids were extracted from a 200-μL sample using the total nucleic acid protocol with the MagNA pure LC nucleic acid isolation system (Roche Diagnostics). Each sample was eluted in 50 μL buffer. Detection of *B. pertussis* and *B. parapertussis* was performed in a multiplex real-time PCR assay, as described elsewhere [17], using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). In brief, samples were assayed in a 25-μL reaction mixture containing 5 μL of DNA, 12.5 μL 2 × TaqMan Universal PCR Master Mix (Applied Biosystems), 300–900 nmol/L of the forward and reverse primers, and 75–200 nmol/L of each of the probes. All samples had been spiked before extraction with an internal control virus (phocine herpes virus) to monitor for efficient extraction and amplification, as described elsewhere [18]. Serological diagnosis of pertussis consisted of measurement of immunoglobulin (Ig) G antibodies against purified pertussis toxin (IgG-Ptx) and IgA antibodies against a crude cell-membrane preparation of *B. pertussis* (IgA-Bp) with an in-house ELISA of the National Institute for Public Health and the Environment. From 2003 onwards, the reference serum
used in this ELISA has been calibrated with the US Food and Drug Administration (FDA) lot 3 international standard serum for Ptx-antibodies. Serum samples were tested in 1:400 dilution (equivalent to 100 FDA lot 3 IU/mL). An IgG-Ptx titer greater than the diagnostic cut-off of 100 U/mL was considered indicative for recent pertussis infection. As assessed by Giammanco et al. [19] this value of 100 FDA lot 3 IU/mL corresponds with 82 “dutch”-U/mL as measured with the reference serum that was in use at our institute before 2003. The specificity of this cut-off was 98.3% when assessed in 7,756 serum samples obtained from the general population and was independent of age [20]. IgA-Bp levels were interpreted in combination with IgG-Ptx levels by constructing height categories of IgA-Bp and IgG-Ptx combinations (height categories 1 to 12, from low to high). The diagnostic cut-off of IgA-Bp-IgG-Ptx combinations was defined to be the 99th percentile of the distribution of IgA-Bp-IgG-Ptx height categories in 7,756 serum samples obtained from the general population. IgA-Bp concentrations in the serum samples obtained from the general population increased with age (the data not shown here but have been demonstrated in previous studies [21, 22]). Consequently, the diagnostic cut-off of IgA-Bp and IgG-Ptx combinations was age-dependent, with separate cutoffs for subjects aged <5 years, 5-14 years, and >14 years. In patients with PCR- or culture-proven pertussis and in control subjects with other respiratory disease, the sensitivity and specificity of those age-dependant cutoffs have been shown to be 80% and 97%, respectively [22].

Case classification and source identification
A household contact was regarded as a confirmed case of pertussis if PCR, culture, or serological tests yielded positive results. The first day of illness was set as the onset of coughing or as the onset of cough-preceding cold symptoms. Cold symptoms occurring >2 weeks before onset of coughing were seen as a separate episode and not related to pertussis infection. A case was considered typical pertussis if it entailed at least 2 weeks of coughing and ≥1 of the following symptoms: paroxysmal coughing, post-tussive vomiting, and/or inspiratory “whooping.” In families, household contacts with laboratory-confirmed pertussis and the index infants were classified according to the chronology of symptom onset as (co)first or (co)second case(s) i.e., (co)first cases were persons with laboratory-confirmed pertussis with earliest date of onset, and (co)second case(s) had laboratory-confirmed pertussis with onset of symptoms at least 1 week (minimum incubation period) after the (co)first case.
Multiple first cases per household could occur if all became sick in the same week. A household case was considered “source case” if onset of symptoms occurred >1 week before the onset of the case in the index infant. Infection of the index infant was assumed to have occurred outside the household if the parents reported contact between the infant and a non-household contact with pertussis infection in the week before onset in the infant.
Statistical analysis
Characteristics of the index infants and household contacts were analysed. Differences in percentages and medians were tested with χ² test, the Fisher exact test, or the Wilcoxon Mann-Whitney test, as appropriate. Linear regression was used to study the relationship between duration of hospital admission, age, and vaccination status of index infants. A P-value <.05 was considered statistically significant. Analyses were performed using SAS software, version 9.1 (SAS Institute). Ninety-five percent confidence intervals were calculated using Episheet [23].

Results
Of 294 reported infants and their families, 90 were excluded because caretakers refused participation (for lack of time or fear of venapuncture) or the index infant lacked laboratory confirmation. Three families were excluded because the index infant had infection with B. parapertussis, leaving 201 infected index infants and their families for further analysis.

Infant index cases
The median number of days between date of hospitalisation of the index infant and the study nurse’s visit to the family was 17 days (range, 4-88 days). All 201 index infants were born in the Netherlands; 104 (52%) were male. The median age at onset of symptoms was 49 days (range, 2-103 days). Twenty-four infants (12%) were born prematurely (<37 weeks gestation period). Table 1 reviews symptoms and complications reported in 201 index infants. All infants survived. The median duration of hospital stay was 8 days (range, 0-80 days); 33 infants (16%) were admitted for day care or treated in the outpatients’ clinic. Among infants eligible for at least 1 vaccination (i.e., aged 56-84 days), the median duration of hospitalisation was shorter in those receiving 1 dose than unvaccinated infants (4 versus 11 days, P=.03).

Attack rate in household contacts
Laboratory diagnostics were performed for 723 (98%) of 738 household contacts: 335 were tested by PCR, serological examination, and culture; 353 were tested by PCR and serological examination; 31 were tested by PCR only, and 4 underwent serological testing only. A positive test result was found for 391 (54%) of the tested persons; 36 (11%) of 335 cases were culture proven, 262 (38%) of 692 were serologically confirmed, and 213 (30%) of 719 were PCR confirmed. In symptomatic, laboratory-confirmed cases, the median duration of symptoms at time of sampling was related to the diagnostic method: cases confirmed by PCR had a shorter duration of symptoms than did serologically confirmed cases (P<.01). To maximize the diagnostic yield and to reliably identify infected contacts, taking into account the delay
Table 1. Clinical symptoms and complications in 201 infants aged <6 months admitted to the hospital for *Bordetella pertussis* infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Infants n=201</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset of symptoms, median days (range)</td>
<td>49 (2-105)</td>
</tr>
<tr>
<td>Doses of vaccine received</td>
<td></td>
</tr>
<tr>
<td>0(^a)</td>
<td>146 (73)</td>
</tr>
<tr>
<td>1</td>
<td>37 (18)</td>
</tr>
<tr>
<td>2</td>
<td>17 (8)</td>
</tr>
<tr>
<td>3</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Comorbidity(^b)</td>
<td>32 (16)</td>
</tr>
<tr>
<td>Duration of cough, median days (range)</td>
<td>30 (3-150)</td>
</tr>
<tr>
<td>Paroxysmal cough</td>
<td>198 (99)</td>
</tr>
<tr>
<td>Number of paroxysms per day, median days (range)</td>
<td>25 (1-240)</td>
</tr>
<tr>
<td>Nonparoxysmal cough</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Cough with whoop</td>
<td>192 (96)</td>
</tr>
<tr>
<td>Posttussive vomiting</td>
<td>147 (73)</td>
</tr>
<tr>
<td>Posttussive sticky sputum</td>
<td>189 (94)</td>
</tr>
<tr>
<td>Wheezing</td>
<td>177 (88)</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>164 (82)</td>
</tr>
<tr>
<td>Apnoea</td>
<td>112 (56)</td>
</tr>
<tr>
<td>Collapse</td>
<td>65 (33)</td>
</tr>
<tr>
<td>Fever</td>
<td>13 (6)</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>12 (6)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>97 (48)</td>
</tr>
<tr>
<td>Convulsions</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Conjunctival infection</td>
<td>36 (18)</td>
</tr>
<tr>
<td>Retinal bleeding</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Otitis media</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Intracranial bleeding</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Administration of oxygen</td>
<td>106 (53)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>8 (4)</td>
</tr>
<tr>
<td>ICU admission</td>
<td>22 (11)</td>
</tr>
<tr>
<td>Death</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Note: data are number (%) of cases unless otherwise indicated. ICU, intensive care unit.

\(^a\) 121 infants were too young to be vaccinated.

\(^b\) Includes other respiratory disorders (respiratory syncytial virus infection, 4 cases; influenza A, 1 case; human metapneumovirus infection, 2 cases; rhinovirus infection, 4 cases; rotavirus infection, 2 cases; and infection with unspecified pathogen, 2 cases); reflux, 13 cases; gastro-intestinal infection, 2 cases; urinary tract infection, 1 case; and damaged pulmonary lobe, 1 case.
Of the 205 children with verified vaccination-status, 180 (89%) had been completely vaccinated in infancy. Within 3 years after completion of the primary series, 9 (29%) of 31 children who received acellular vaccine had typical pertussis, compared with 17 (46%) of 37 children who received whole-cell (Table 3). Of the unvaccinated children aged 1-3 years, 4 (67%) had typical pertussis.

The twin brother of 1 index infant was hospitalised for pertussis for 9 days. Patients hospitalised for 1 day included 1 sibling (a 7-year-old child who had been vaccinated according to schedule, including the acellular preschool booster) and 1 father (age, 44 years). Five household contacts with pertussis reported having a diagnosis of pertussis confirmed by a general practitioner (GP) in the past (3-27 years ago, at the time that the subject was aged 0-4 years).

### Table 2. Attack rates of *Bordetella pertussis* infection, by age and disease manifestation, in household contacts of infants hospitalised for pertussis.

<table>
<thead>
<tr>
<th>Household contact</th>
<th>Number of persons</th>
<th>Disease manifestation, no. (% of persons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Typical</td>
</tr>
<tr>
<td>Mothers</td>
<td>164</td>
<td>58 (35)</td>
</tr>
<tr>
<td>Fathers</td>
<td>155</td>
<td>29 (19)</td>
</tr>
<tr>
<td>Other adults*</td>
<td>28</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Adolescents aged 14-19 years</td>
<td>8</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Siblings aged 9-13 years</td>
<td>85</td>
<td>22 (26)</td>
</tr>
<tr>
<td>Siblings aged 1-4 years</td>
<td>92</td>
<td>32 (35)</td>
</tr>
<tr>
<td>Siblings aged 0 years (i.e., twins)</td>
<td>1</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>560</td>
<td>159 (28)</td>
</tr>
</tbody>
</table>

* Seventeen of 28 were grandparents

Of the 205 children with verified vaccination-status, 180 (89%) had been completely vaccinated in infancy. Within 3 years after completion of the primary series, 9 (29%) of 31 children who received acellular vaccine had typical pertussis, compared with 17 (46%) of 37 children who received whole-cell (Table 3). Of the unvaccinated children aged 1-3 years, 4 (67%) of 6 had typical pertussis.

The twin brother of 1 index infant was hospitalised for pertussis for 9 days. Patients hospitalised for 1 day included 1 sibling (a 7-year-old child who had been vaccinated according to schedule, including the acellular preschool booster) and 1 father (age, 44 years). Five household contacts with pertussis reported having a diagnosis of pertussis confirmed by a general practitioner (GP) in the past (3-27 years ago, at the time that the subject was aged 0-4 years).
Table 3. Number of pertussis cases per disease manifestation, in relation to time since last vaccine dose for children who were completely vaccinated (i.e., children who had received 4 doses) with either the whole-cell vaccine or the acellular vaccine but who did not receive a preschool booster.

<table>
<thead>
<tr>
<th>Vaccine regimen received, duration since last dose</th>
<th>total</th>
<th>typical pertussis</th>
<th>atypical pertussis</th>
<th>asymptomatic pertussis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Four doses of whole-cell vaccine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 years</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 years</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2 years</td>
<td>25</td>
<td>11</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3 years</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4 years</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥5 years</td>
<td>38</td>
<td>16</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Four doses of acellular vaccine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 years</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1 years</td>
<td>15</td>
<td>4</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>2 years</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3 years</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

In total, 116 (39%) of 299 contacts with current pertussis infection consulted a GP for their infection. The GPs had ordered laboratory tests for 23 (20%) and treated 11 (9%) before onset in the index infant. Ten infected adult household contacts (5%) lost work days (median, 2 days; range, 1-10 days) because of their pertussis.

Introduction of pertussis in the households and transmission to neonates

Table 4 shows the distribution of first cases in the households. 93 (48%) had typical pertussis. Of the first cases, 28% were siblings, 24% mothers, and 11% fathers. Of the mothers, 14 (22%) of 46 had onset of symptoms during pregnancy. The index infant was the first case in 68 households, but in 11 infants (7%), the onset of symptoms coincided with onset of symptoms in a household contact. One index infant (0.6%) had been exposed to a child outside the household whose pertussis was diagnosed in the week before onset of symptoms in the index infant. When the index infant was the second, third, or fourth case in a household, 41% of the source cases were siblings, 38% were mothers, and 17% were fathers.
Table 4. Classification of first cases and source cases (i.e., household contacts with onset ≥ 7 days preceding the index infant), by relationship with the infant.

<table>
<thead>
<tr>
<th>Relationship with index infant</th>
<th>First cases</th>
<th>Source cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of persons</td>
<td>No. of persons</td>
</tr>
<tr>
<td>Index infant</td>
<td>164</td>
<td>68</td>
</tr>
<tr>
<td>Mother</td>
<td>164</td>
<td>46</td>
</tr>
<tr>
<td>Father</td>
<td>155</td>
<td>21</td>
</tr>
<tr>
<td>Other adult</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>Adolescent aged 14-19 years</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Sibling aged 9-13 years</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Sibling aged 5-8 years</td>
<td>85</td>
<td>17</td>
</tr>
<tr>
<td>Sibling aged 1-4 years</td>
<td>92</td>
<td>25</td>
</tr>
<tr>
<td>Sibling aged 0 years (i.e., twins)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>560</td>
<td>192</td>
</tr>
</tbody>
</table>

* Multiple first cases per household could occur.

Discussion

Currently in the Netherlands, an infant hospitalised for pertussis has most often been infected in the home by siblings or mother (in 33% and 28% of the cases, respectively). Of the possible sources of infection of the index infant (i.e., contacts with onset of symptoms at least 1 week before onset in the index infant), 41% were siblings, 38% were mothers, and 17% were fathers.

Our finding that mothers play an important role in the transmission of pertussis in the household is consistent with previous studies [11, 12, 15]. Fathers have less importance in pertussis transmission, especially in the first 3 months of life, presumably because only mothers receive pregnancy leave in the Netherlands.

In contrast to other studies [14, 15], we found that siblings were the main source of infection in infants. The distribution of infection in contacts may reflect the demographic characteristics and family structures in the study households and is also influenced by vaccination history [24], characteristics of the circulating pathogen [6] and country-specific contact patterns [25]. The mean number of children (2.6; 95% confidence interval, 2.3-2.8) in the study households exceeded that of the general population in 2007 (1.8) [26], suggesting that having siblings is a risk factor for infant pertussis [27]. Alternatively, it could indicate that households with several children are over-represented in our analyses, resulting in overestimation of sibling’s
importance. However, unlike previous studies, we included >80% of the infants hospitalised nationwide for pertussis in the study period, and our final analyses – with both PCR and serology results available for all household contacts – was based on 164 families. Therefore, our study population seems to be representative and yields a robust estimation of the role of various household contacts in the transmission of pertussis to infants.

The larger role of siblings in the transmission of pertussis in the Netherlands may result from using a less effective whole-cell vaccine [28]. In areas with low vaccine coverage, young children most often introduce pertussis into a household [10], whereas in high-coverage areas adolescents and adults play a larger role [7, 29]. Despite high uptake in the 1990s, the relatively less effective Dutch whole-cell vaccine may have created an area with low immunity. The lower effectiveness of the Dutch whole-cell vaccine is underlined by the fact that 44% of completely vaccinated children got typical pertussis. However, even among children completely vaccinated with the acellular vaccine (introduced in 2005), 29% got typical pertussis within 3 years after completion of the primary series of vaccination. Conceivably, intra-familial factors (such as host genetics or immunological background) may induce vaccine failure [30], and prolonged and intense exposure (as in a household) may overcome vaccine-induced protection. Besides, 71% of siblings in the current study had not received the acellular preschool booster, introduced in 2001. The role of siblings in the transmission of pertussis may diminish in the coming years as introduction of the preschool booster reduced the incidence of pertussis in infants, probably due to reduced transmission from siblings [5].

In one-third of the cases, we found no household contact with symptom onset preceding the infection in the index infant, although most households had ≥1 contact shown to be infected. It could be that contacts were asymptomatic, failed to recall symptoms, or that symptoms were slow to develop. Asymptomatic or subclinical infections may still transmit pertussis to vulnerable infants [9, 13]. Of course, the source of infection could have been a casual contact outside the household [31].

Pertussis may run a severe course in infants, especially those who are prematurely born, and hospitalisation may exceed 1 week [7, 12, 32, 33]. Fortunately, none of the infants in the study died or had severe complications (e.g., encephalopathy and intracranial bleeding), although we cannot exclude the possibility that those of non-participating parents may have been more severely ill. Most index infants in the study were too young to have been completely vaccinated. However, protection against severe pertussis can be achieved after one dose of vaccine [34, 35], and duration of hospitalisation – a marker for severity – was significantly lower among infants who had received 1 dose.

Although most household contacts were vaccinated, the attack rate of symptomatic pertussis was high (46%). In a similar study in a highly vaccinated population in France [7], the attack
rate of symptomatic pertussis in household contacts was equally high, although not all cases were laboratory confirmed. We showed that only 39\% of infected household contacts consulted a GP, and 20\% were tested. Importantly, only 9\% were treated with antibiotics before onset of symptoms in the index infant, conforming to the protocol for prophylaxis to limit secondary spread to infants [36]. Only 5\% of infected adults reported loss of working days because of disease. Thus, most pertussis infections beyond infancy are mild and go unnoticed by the health care system. In the short term, a number of other measures could be implemented to protect infants.

First, the vaccination schedule could start at 6 weeks of age [37], which theoretically would have prevented severe disease in almost 40\% of the cases in this study. In fact, if proven safe, effective, and accepted by the population, vaccination directly after birth [38] or vaccination of mothers during pregnancy [39] could protect infants even in the first weeks of life.

Second, selective vaccination of new parents will reduce transmission to infants. In 35\% of our study households, pertussis was introduced by a parent, and parents accounted for 55\% of the source cases. Instead of selective vaccination of parents, overall adult vaccination has been suggested [40] and is recommended in some countries. However, since adult vaccination has so far obtained low coverage [41] and is unlikely to be cost-effective [42], this strategy is not promising. Because expectant parents have regular contact with health care and are well motivated to protect their child, their selective vaccination needs consideration. In the long term, pertussis vaccines and vaccination strategies should be improved to give longer protection against both disease and infection.

Acknowledgements

We thank all participating families and all paediatricians, microbiologists, and local public health services who reported infants for the study.
References


Vaccination of young parents who are in close contact with infants ("cocooning strategy") will reduce transmission of pertussis to infants.

Picture obtained from folder entitled "Bescherm uw baby tegen kinkhoest", a publication of the "Vlaams Agentschap Zorg en Gezondheid" within the scope of the European Vaccination week 2009.
The case for maternal vaccination against pertussis

Frits R. Mooi
Sabine C. de Greeff

Lancet Infect Dis 2007;7:614-624
Abstract
Despite high vaccine coverage pertussis is increasing in a number of countries. Particularly alarming is the increase of pertussis in infants too young to be (fully) vaccinated, because the highest morbidity and mortality is observed in this category. Maternal vaccination offers the possibility to protect infants from birth until immunity is induced by active vaccination and has been shown to be effective and safe for tetanus over long periods of time. Maternal vaccination studies with whole-cell pertussis vaccines have not shown serious adverse effects in mother and child. In one study, protection of newborn babies was found. Additional support for the efficacy of maternal vaccination comes from studies showing that transfer of antibodies confers protection against pertussis. Maternal vaccination might be an effective way to decrease morbidity and mortality caused by pertussis in newborn babies.
Introduction
Although pertussis vaccines have been used in many countries since the 1940s and 1950s, the disease has remained endemic. Furthermore, in the 1980s and 1990s, a resurgence of pertussis was observed in a number of countries despite high vaccination coverage [1-5]. Several explanations have been put forward for this phenomenon including improved surveillance, waning immunity, bacterial evolution and the use of subpotent vaccines [6]. The relevance of these factors in the resurgence of pertussis might differ between countries - for example, the use of a subpotent vaccine had an important role in Canada, whereas both a subpotent vaccine and strain evolution were important in the Netherlands [1, 3]. The resurgence of pertussis has resulted in an increased morbidity and mortality in infants too young to be vaccinated. In several European countries, infants obtain their primary vaccination at the ages of 2, 3, and 4 months [7]. Australia, Canada, and the USA follow a schedule of primary vaccination with doses at 2, 4, and 6 months. Assuming that acceptable immunity is reached 1 month after the third injection [8], this leaves infants younger than 5-7 months at least partially susceptible to pertussis. It is in this age category that the highest morbidity and mortality is observed [9].

Morbidity and mortality in infants caused by pertussis
In the Netherlands, 416 out of 756 (55%) infants less than 6 months of age and reported with pertussis were hospitalised in 2000-04. In the past decade, nine deaths caused by pertussis were registered in the Netherlands, all in children younger than 3 months of age. In the USA, 1567 out of 2488 (63%) infants less than 12 months of age reported with pertussis were hospitalised in 2000-04 [10]. Of the 100 pertussis-related deaths reported in the USA in this period, 90 (90%) were in infants aged less than 4 months and 76 (76%) were in infants aged less than 2 months [10]. Also, in the UK the morbidity of reported pertussis is highest among infants less than 2 months of age [11]. Despite high vaccination rates, the incidence of reported pertussis in infants younger than 12 months of age in the USA increased by 49%, from 34.2 per 100,000 population in the 1980s to 51.1 per 100,000 population in the 1990s. This increase was predominantly caused by the increase of disease in infants less than 4 months of age [12]. Increasing rates of infant pertussis have also been documented in Canada and several European countries [13, 14]. Moreover, the number of deaths caused by pertussis in infants younger than 4 months of age is also increasing. One study showed that the reported number of deaths caused by pertussis in this age group in 1980-1989 and 1990-1999 in the USA were 49 and 84, respectively, an increase of 71% [15]. In line with the observations in the USA, the incidence of pertussis in infants in the Netherlands
increased 2.8-fold in the period 1996-2004 compared with 1989-95. The incidence of hospitalisations in infants increased by 82%, from 66.3 per 100,000 population in the period 1989-1995 to 121.0 per 100,000 population in 1996-2004 (Table 1) [1, 16]. The number of deaths in the two periods was two and nine, respectively. All deaths occurred in infants less than 3 months of age, except for one death in 1993 in the 5-9-year age group. Since routine vaccination is scheduled at 2, 3, and 4 months in the Netherlands, most of these children were too young to be protected directly by vaccination.

Presumably, the number of infant cases of pertussis is higher than routine surveillance data report because pertussis in infants might be atypical and therefore not recognized [9, 17, 18]. A case report from the UK demonstrated that out of five infants less than 3 months old who were admitted to hospital for pertussis, none showed the characteristic whoop or paroxysmal cough, and one of the infants did not cough at all [19]. Application of the capture-recapture method to assess the completeness of reporting to the national surveillance system in the USA indicated that more than two-thirds of all pertussis deaths were not accounted for in the national disease reporting system [20]. A similar study in the UK concluded that mortality of pertussis in infants is underestimated by 60% in the national death register [21]. Another study in the UK showed that morbidity in infants was also underestimated: pertussis was clinically suspected on admission in only 28% of the infants who were later diagnosed with the disease [22].

**Table 1.** Incidence of hospitalisations per 100,000 for pertussis by age group in the Netherlands in 1989-1995 compared with 1996-2004.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>0 years</td>
<td>66.3</td>
<td>121.0</td>
<td>1.8</td>
</tr>
<tr>
<td>1-4 years</td>
<td>4.9</td>
<td>6.8</td>
<td>1.4</td>
</tr>
<tr>
<td>5-9 years</td>
<td>1.3</td>
<td>2.59</td>
<td>2.0</td>
</tr>
<tr>
<td>10-14 years</td>
<td>0.4</td>
<td>0.54</td>
<td>1.4</td>
</tr>
<tr>
<td>15-19 years</td>
<td>0.03</td>
<td>0.09</td>
<td>3.0</td>
</tr>
<tr>
<td>More than 19 years</td>
<td>0.02</td>
<td>0.07</td>
<td>3.5</td>
</tr>
<tr>
<td>All ages</td>
<td>1.21</td>
<td>2.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* The incidence for both periods (1989-1995 and 1996-2004) was calculated as (number of hospitalised cases in the concerning period × 100,000)/population at risk in that period. Data taken from references [1] and [16].
Vaccination strategies to reduce pertussis in infants

Different vaccination strategies can be implemented to protect infants against pertussis. Protection of unvaccinated or partly vaccinated infants can be enhanced, indirectly, by decreasing the circulation of *Bordetella pertussis*. Universal vaccination of 4-year-olds, adolescents, adults, or selective immunization of new mothers and fathers (cocooning) not only protects the vaccinated group, but increases herd immunity and hence reduces disease transmission to unvaccinated or incompletely vaccinated infants. Several studies have provided evidence that this approach might be successful. The introduction of a booster vaccination for 4-year-old children in the Netherlands reduced the number of hospitalisations among infants less than 4 months of age [23]. In Canada, introduction of an adolescent booster vaccination not only resulted in a decrease in the pertussis incidence in this age group, but also in infants less than 12 months of age [24]. In Australia, a small decrease in hospitalisations in children aged 3-4 months was observed after the introduction of a booster given to children of 4 years of age. However, no change in the 0-2 month age group was reported [25, 26].

Direct protection of the infant may be conferred by maternal and neonatal vaccination. Compared with neonatal vaccination, maternal vaccination has several advantages. First, maternal vaccination offers the possibility to protect the infant from birth until immunity is achieved by active vaccination. By contrast, neonatal vaccination will leave the infant susceptible to pertussis for a period of weeks to months depending on how fast immunity is induced. An advantage of both maternal and neonatal vaccination is the accessibility of the targeted group, since both (pregnant) mothers and infants frequently visit health-care centres.

Maternal immunity

Maternal vaccination confers protection by efficient placental transfer of maternal antibodies [27, 28]. Maternal memory T cells and B cells are ineffective because of differences in tissue antigens (HLA in particular) between the mother and her foetus [27]. Furthermore, there is no evidence that immune cells are able to cross the placental barrier and establish themselves in the foetus. The time period in which maternal vaccination is effective depends on the timing of the mother’s immune response to the vaccine, and also the timing of maternal-foetal IgG transport [29, 30]. Foetal IgG remains low until the second trimester and increases during the third trimester. Indeed, the rate of increase in foetal IgG concentration between 29 and 41 weeks of gestation is roughly twice that seen between 17 and 28 weeks [31]. For these reasons, and also in view of real or apparent side-effects, the optimum timing for maternal vaccination is in the third trimester, probably between weeks 30 and 32 [28]. The IgG subclasses are transported with different efficiency according to the following sequence: IgG1 (most efficient), IgG4,
IgG3, IgG2 (least efficient) [31]. Thus it is important to determine the predominant antibody subclass induced by maternal vaccination, because this will determine the efficacy in the child. Since transmission of maternal antibodies reaches its maximum during the last few weeks of pregnancy, premature infants may be deficient in protective antibodies. However, maternal vaccination offers additional advantages for breastfed infants who passively acquire IgA antibodies from breast milk. Protection of infants in this manner has been demonstrated for both gastrointestinal and respiratory pathogens [32]. High IgA titres in sera from infants younger than 1 year of age were found to correlate with reduced duration of positive pertussis culture and PCR in throat samples [33]. These findings point to bactericidal effects of anti-B. pertussis IgA in human beings. Bactericidal effects of anti-B. pertussis IgA antibodies have been observed in transgenic mice [34].

Maternal vaccination

The usefulness of maternal vaccination was already recognized more than 120 years ago. In 1879, Burkhardt [35] reported that infants born to mothers who had “Jennerian vaccination” during pregnancy were protected from the vaccinia virus during the first days of life. In 1892, Ehrlich demonstrated the protective function of maternally transferred antibodies against microbial infections in mice offspring [36]. Furthermore, in the early 20th century it became clear that certain common infectious diseases such as diphtheria, poliomyelitis, scarlet fever, and measles did not manifest themselves in the first few months of life because of passively acquired antibodies from the immune mother [37, 38]. It was also noted in this period that other infectious diseases attack the neonate and young infant because they failed to acquire antibody from mothers, who had either no immunity or low levels of antibodies. More recent studies have confirmed these observations by showing that the occurrence of some neonatal infections - caused by group B streptococci, Haemophilus influenzae type b (Hib), and respiratory syncytial virus - is correlated with low maternal or neonatal antibody levels [39-41].

These insights stimulated attempts to protect newborn babies by maternal vaccination. For only a few diseases this has led to the incorporation of maternal vaccination in the routine vaccination programme, tetanus vaccination being the most prominent example [28, 42]. One of the reasons why maternal vaccination has not been implemented more widely is the initial success of infant vaccination, which has substantially reduced morbidity and mortality in childhood. More recently, liability issues have hampered the application of maternal vaccination [43].
It is noteworthy that the success of vaccination programmes has led to a reassessment of the usefulness of maternal vaccination. Because of mass vaccination, the circulation of many pathogens has decreased, leading to diminished population immunity as a result of lack of natural boosters. Consequently, most women in childbearing age now have low levels of antibodies against childhood diseases. Reduced levels of neutralizing antibodies during pregnancy influence the competence of transferred maternal immune protection, leading to a larger window of susceptibility in newborn babies. The latter could result in an increase of childhood infections and an exacerbation of infections that are usually mild. Currently, vaccines with the highest potential benefit in reducing childhood illness by maternal vaccination are those for respiratory syncytial virus, *Streptococcus pneumoniae*, group B streptococci, *B. pertussis*, and parainfluenza virus type 3 [28, 44].

**Transfer and persistence of maternal antibodies**

Preferably, maternal vaccination should completely close the window of susceptibility in the infant by providing immunity until this is replaced by active vaccination. A German study assessed the effectiveness of complete and partial pertussis vaccination for the prevention of severe pertussis requiring hospitalisation in 529 patients [8]. The vaccination schedule was 2, 3, and 4 months and for most doses (89%) a three-component acellular vaccine was used. Vaccine efficacy was 68%, 92%, and 100% for one, two, and three doses, respectively. Thus, ideally, maternal vaccination should provide protection until the third vaccination is given – i.e., during the first 4-5 months after birth.

In one study the average half-life of human maternal IgG1 was found to be 48.4 days in infants [45]. However, the persistence of protective levels of maternal antibodies differs widely depending on, for example, initial levels, specificity and avidity. Meningococcus type A or C and Hib antibodies remained at protective levels for 3 months and more than a year, respectively, after maternal vaccination [32]. The levels of *B. pertussis*-specific antibodies required for protection are not known. Active placental transfer of pertussis antibodies has been observed resulting in higher concentrations in the newborn baby compared with the mother [46]. Transplacental pertussis antibody concentrations in newborn babies were found to decline with a half-life of around 6 weeks and by the age of 2-6 months most infants had no detectable antibodies to *B. pertussis* [46-49].
Interference of maternal antibodies with vaccination of the child

A major concern associated with maternal vaccination is interference with the childhood vaccination, because pre-existing antibodies gained by placental transfer might affect the infant’s immune response to primary immunization. It is now assumed that the primary cause for maternal interference is epitope masking by maternal antibodies, preventing antigen binding by infant B cells [50]. The inhibitory effect is dependent on the ratio of maternal antibodies at the time of vaccination and the dose of antigen used to vaccinate the infant. Several studies have addressed the issue of maternal interference in pertussis vaccination. Maternally derived antibodies have been shown to interfere with antibody responses when whole-cell vaccines were used, but not, or much less, when acellular vaccines were used in the infant [48, 51, 52]. The latter can probably be attributed to the higher amounts of antigens present in acellular vaccines. However, in these studies the maternal population was immunized by infection or vaccination with a whole-cell vaccine and, since maternal immunization with acellular vaccines might induce higher levels of antibodies, it may show a higher degree of interference with infant immunization.

Evidence for maternal immunity against pertussis

Ethical, technical, and legal dilemmas complicate the possibility of undertaking efficacy trials, hence recent information on the efficacy of maternal vaccination and its possible risks is limited. Consequently, it is important to review the currently available evidence that maternal immunity is effective against pertussis in neonates. In the prevaccination era, it was common knowledge that, although newborn babies had a high degree of resistance to childhood diseases such as measles, poliomyelitis, scarlet fever, and diphtheria, they appeared to be susceptible to pertussis from the day of birth [37, 53]. This led to the idea that maternal immunity against pertussis was ineffective. However, sero-epidemiological studies in this period indicated that only 14-34% of the pregnant women had detectable antibodies to pertussis [54]. This suggests that the relative high incidence of pertussis in newborn babies compared with other childhood diseases is because of lack of booster infections of mothers, or because of the fact that immunity to pertussis wanes faster compared with other childhood diseases. In a recent study, lack of maternal antibodies has been proposed to be a risk factor for infant pertussis [55].
Role of antibodies in pertussis immunity

Maternal immunity is based on passively acquired antibodies [29], in particular IgG1 (see above); thus it is important to establish whether antibodies alone suffice for protection of the newborn against pertussis. There is ample evidence that this is the case, as discussed below.

Antibody titres against pertussis antigens are correlated with protection in human beings

Early studies indicated that, although immunity might exist in the absence of agglutinins (antibodies that agglutinate bacteria), susceptibility is not observed in the presence of high titres of agglutinins [56, 57]. During field trials with whole-cell vaccines in the UK in the 1950s, it was also shown that agglutinin titres correlated with protection against pertussis in children [58]. Later, it was shown that agglutinating antibodies are mainly directed against fimbriae [59]. More recently, levels of antibodies against filamentous haemagglutinin, pertussis toxin, pertactin, fimbriae, and lipopolysaccharide have been associated with protection against pertussis [60-66]. However, correlation does not imply causation, and the antibody titres against distinct antigens might reflect other immune mechanisms important for protection, such as memory and cellular immunity, or even an overall elevated antibody level against B. pertussis antigens. Evidence for a direct role of antibodies in pertussis immunity has been obtained from passive vaccination studies in animals and human beings.

Passive vaccination studies in animals

Results from animal models used to study immunity to B. pertussis should be interpreted carefully, because the disease differs substantially between animals and human beings. Nevertheless, important relations have been found between vaccine efficacies established in human beings and mice [58, 67, 68]. There is substantial evidence that transfer of antibodies can confer protection in animals (Table 2). The antibodies used for passive vaccination were raised against specific antigens, some of which are part of the current acellular vaccines (e.g., pertussis toxin, filamentous haemagglutinin, and pertactin), or whole bacteria. In mice, passive vaccination has been shown to reduce colonisation, loss in bodyweight, leucocytosis, and number of deaths. Particularly relevant are experiments performed by Huang and colleagues [69] in which monkeys were passively vaccinated with serum from infected or vaccinated monkeys. Three out of four monkeys treated with convalescent serum did not develop pertussis, whereas one out of two monkeys injected with serum from vaccinated monkeys developed disease. Control animals developed a disease very much like pertussis, except that whoops were absent.
Passive vaccination studies in human beings

Prophylactic and therapeutic treatment of pertussis by passive vaccination has a long history and the first attempts, often with a few infants, were made in the early 1900s [70, 71]. In 1923, Debré [72] passively vaccinated 40 children who had intimate contact with a child with pertussis. Of these 40 children, 31 did not develop pertussis, whereas six children had pertussis in an attenuated form. Three children developed normal pertussis. Many studies followed, which were summarised by Bradford in 1935 [73].

Table 2. Passive vaccination studies in animals.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Source antibodies</th>
<th>Animal model</th>
<th>Challenge</th>
<th>Outcomeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al. (1962) [69]</td>
<td>Whole-cell vaccine</td>
<td>Monkey</td>
<td>Monkey</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Sato et al. (1981) [120]</td>
<td>Filamentous haemagglutinin or pertussis toxin</td>
<td>Rabbit</td>
<td>Mouse</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Sato et al. (1984) [121]</td>
<td>Pertussis toxin</td>
<td>Monoclonal antibody</td>
<td>Mouse</td>
<td>Intracerebral, aerosol</td>
</tr>
<tr>
<td>Sato and Sato (1985) [95]</td>
<td>Filamentous haemagglutinin or pertussis toxin</td>
<td>Mouse</td>
<td>Mouse</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Oda et al. (1985) [122]</td>
<td>Diverse</td>
<td>Human colostrum</td>
<td>Mouse</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Montaraz et al. (1985) [123]</td>
<td>Pertactin b</td>
<td>Monoclonal antibody</td>
<td>Mouse</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Kimura et al. (1990) [124]</td>
<td>Filamentous haemagglutinin</td>
<td>Goat, rat</td>
<td>Mouse</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Olander et al. (1990) [125]</td>
<td>Whole cells, filamentous haemagglutinin, pertussis toxin</td>
<td>Mouse</td>
<td>Mouse</td>
<td>Intranasal</td>
</tr>
<tr>
<td>Shahin et al. (1990) [126]</td>
<td>Pertactin</td>
<td>Monoclonal antibody</td>
<td>Mouse</td>
<td>Aerosol</td>
</tr>
<tr>
<td>King et al. (2001) [127]</td>
<td>Pertactin</td>
<td>Monoclonal antibody</td>
<td>Mouse</td>
<td>Intranasal</td>
</tr>
<tr>
<td>Hellwig et al. (2003) [128]</td>
<td>Whole-cell vaccine</td>
<td>Rabbit</td>
<td>Mouse</td>
<td>Intranasal</td>
</tr>
</tbody>
</table>

a Outcome of challenge in vaccinated animals, relative to control animals.
b Derived from Bordetella bronchiseptica. Challenge with a B. bronchiseptica strain.
c With B. bronchiseptica.
Bradford concluded “it seems probable that immune blood is effective in the prevention and modification of pertussis if given before the catarrhal symptoms appear. If given after the disease is established favourable results are less apparent”. Two important improvements in passive vaccination were the use of more defined, hyperimmune serum from recently vaccinated adults [74] and the lyophilisation of serum [75]. The latter allowed stable storage and two to four-fold concentration of serum. In the period 1935-47, additional studies were carried out to assess the effect of passive vaccination against pertussis. Lack of (untreated) controls makes it difficult to interpret the results. Both promising [75-87] and negative [88-91] results were obtained with passive vaccination.

Different outcomes were caused by several factors. The potency of the sera used probably differed extensively – e.g., sera were used from adults without a known history of pertussis, adults whom had pertussis in childhood, and adults who had recent pertussis. The doses applied varied widely, from 10-140 mL. Importantly, infants were treated in different stages of the disease and the length and intensity of exposure after treatment was often not well defined. To correct for these factors, untreated controls are essential.

A number of studies in which infants were treated before any evidence of clinical disease and that included untreated controls are shown in Table 3. Compared with the untreated infants the efficacy of passive vaccination varied between -34% and 88%. Only one study did not find a positive effect of passive vaccination [89]. In this study, susceptibility for disease was assessed for 28 days after passive vaccination and it is conceivable that protection conferred by the passively acquired antibodies did not last that long.

In more recent times, Granstrom and colleagues [92] investigated the therapeutic effect of high titre, hyper-immune human serum raised with a monocomponent or a two-component acellular vaccine (with, respectively, pertussis toxin and pertussis toxin/filamentous haemagglutinin). The control consisted of a 20% albumin solution. The main finding was a significantly shorter duration of whoops in the treated group compared with the control group. Duration of whoops post-treatment was 8.7 days (95%CI: 4.8-12.6) in the 33 children receiving immunoglobulin versus 20.6 (95%CI: 11.9-29.3) in the 14 receiving placebo (P=0.0041). Early therapy was important, since the duration of whoops was shorter in children with less than 7 days' disease duration before treatment than in those with disease duration of 8-14 days. Passive vaccination did not decrease the duration of vomiting or coughs that were not followed by whooping.

Bruss and colleagues [93] investigated the therapeutic effect of a 4% IgG solution of pooled plasma from donors immunized with inactivated pertussis toxoid for passive vaccination of infants (26 patients, mean age 9.7 weeks). No control group was included. Improvements in paroxysmal coughing, desaturations, bradycardic episodes, and a decline in lymphocyte count
Table 3. Efficacy of passive vaccination in exposed infants.

<table>
<thead>
<tr>
<th>Ref</th>
<th>Group size</th>
<th>Age (number)</th>
<th>Serum¹</th>
<th>Volume²</th>
<th>Injections</th>
<th>No. disease</th>
<th>Disease</th>
<th>Efficacy³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradford (1935) [73]</td>
<td>27</td>
<td>0-3 yrs (24), &gt;3 yrs (3)</td>
<td>Normal adult and convalescent</td>
<td>6-10 mL</td>
<td>One</td>
<td>12 (44%)</td>
<td>15 (56%)</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>&gt;3 yrs (17)</td>
<td>Untreated</td>
<td>...</td>
<td>0</td>
<td>20 (100%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Paterson et al. (1935) [86]</td>
<td>65</td>
<td>1 month to 4 yrs</td>
<td>Convalescent</td>
<td>10 mL</td>
<td>One</td>
<td>40 (42%)</td>
<td>55 (58%)</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>1 month to 4 yrs</td>
<td>Untreated</td>
<td>...</td>
<td>6 (11%)</td>
<td>49 (89%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Meehan (1937) [81]</td>
<td>115</td>
<td>0-6 yrs</td>
<td>Convalescent</td>
<td>10 mL</td>
<td>One</td>
<td>78 (67%)</td>
<td>37 (32%)</td>
<td>51%</td>
</tr>
<tr>
<td></td>
<td>183</td>
<td>1-6 yrs</td>
<td>Untreated</td>
<td>...</td>
<td>62 (34%)</td>
<td>121 (66%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Schermerhorn (1938) [82]</td>
<td>30</td>
<td>&lt;5 yrs</td>
<td>Convalescent and 20 mL and 10 mL</td>
<td>Two (spacing 10 days)</td>
<td>22 (73%)</td>
<td>8 (27%)</td>
<td>68%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>NR</td>
<td>Untreated</td>
<td>...</td>
<td>5 (17%)</td>
<td>24 (82%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Schermerhorn (1938), Kendall (1939) [82, 83]</td>
<td>11</td>
<td>&lt;1 yr</td>
<td>Hyperimmune</td>
<td>10 mL</td>
<td>Two (spacing 10 days)</td>
<td>9 (82%)</td>
<td>2 (18%)</td>
<td>77%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>&lt;1 yr</td>
<td>Untreated</td>
<td>...</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Cohen and Lapin (1939) [84]</td>
<td>13</td>
<td>Infants</td>
<td>Adult serum 20 or 40 mL</td>
<td>One</td>
<td>8 (62%)</td>
<td>5 (38%)</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Infants</td>
<td>Hyperimmune 10 or 20 mL</td>
<td>One</td>
<td>8 (67%)</td>
<td>4 (33%)</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Infants</td>
<td>Convalescent 15-40 mL</td>
<td>One</td>
<td>28 (85%)</td>
<td>5 (15%)</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Infants</td>
<td>Untreated</td>
<td>...</td>
<td>6 (30%)</td>
<td>14 (70%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Silvertone and Brown (1942) [85]</td>
<td>25</td>
<td>2 months to 14 yrs</td>
<td>Hyperimmune rabbit</td>
<td>5-10 mL</td>
<td>One</td>
<td>24 (96%)</td>
<td>1 (4%)</td>
<td>88%</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>2 months to 14 yrs</td>
<td>Untreated</td>
<td>...</td>
<td>18 (82%)</td>
<td>7 (32%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Morris and McDonald (1957) [89]</td>
<td>17</td>
<td>&lt;5 yrs</td>
<td>Hyperimmune, IgG fraction 2.5 mL (i.e., 25 mL serum)</td>
<td>One</td>
<td>7 (41%)</td>
<td>10 (59%)</td>
<td>-34%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>&lt;5 yrs</td>
<td>Untreated</td>
<td>...</td>
<td>9 (56%)</td>
<td>7 (44%)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&lt;5 yrs</td>
<td>Hyperimmune, IgG fraction 6 mL (i.e., 80 mL serum)</td>
<td>One</td>
<td>2 (25%)</td>
<td>6 (75%)</td>
<td>-13%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>&lt;5 yrs</td>
<td>Untreated</td>
<td>...</td>
<td>3 (33%)</td>
<td>6 (67%)</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

NR=not reported, …=not applicable

¹ Convalescent, serum from patients recovered from pertussis. Adult, serum from adults without recent pertussis. Hyperimmune, serum from adults or rabbits vaccinated with pertussis vaccine.

² The 2.5 mL and 6 mL IgG fractions represent the equivalent of 25 mL and 90 mL serum, respectively.

³ Efficacy was calculated as follows: (number of expected cases-number of observed cases)/number of expected cases. The number of expected cases was based on the number of cases found in the untreated control groups.

⁴ Controls consisted of infants vaccinated prophylactically, which was not effective.
were observed after infusion of the IgG solution, which was well tolerated. In a subsequent phase III clinical trial (25 patients, mean age 9 weeks), no difference was observed in the number or rate of improvement of symptoms (paroxysmal cough, whoop, apnoea, bradycardia, oxygen desaturations) in IgG recipients compared with placebo [94]. However, the trial was prematurely terminated because of expiration of the IgG lots and unavailability of patients.

In summary, studies in animals indicate that immunity against pertussis can be transferred by antibodies. Studies in human beings are confounded by many factors, but when passive vaccination was given before symptoms (i.e., prophylactically), protection was generally observed. By contrast, when passive vaccination was given to infants with symptoms (i.e., therapeutically), little or no effect was found.

Maternal vaccination against pertussis

Animal studies

A number of studies in animals support the efficacy of maternal vaccination against pertussis. Several studies showed that suckling mice were protected against an aerosol challenge with *B. pertussis* when pregnant mice were immunized with pertussis vaccines or their components [95, 96]. Pertactin, a component of most acellular vaccines, derived from *Bordetella bronchiseptica*, an animal pathogen closely related to *B. pertussis*, was used to vaccinate pregnant sows [97]. After challenge with *B. bronchiseptica*, all 19 (100%) control piglets from unimmunized sows developed pneumonia, coughing, and sneezing, and 14 (74%) of the animals developed severe atrophic rhinitis. In 12 piglets from a sow immunized with pertactin, pneumonia occurred only in 34% of the offspring, coughing was reduced, the duration of coughing bouts was shortened, and severe atrophic rhinitis occurred in only one animal (8%). The difference in the occurrence of atrophic rhinitis and pneumonia in offspring of immunized and non-immunized mothers was significant (P<0.05). More recently, transfer of maternal immunity through colostrum was shown in sows vaccinated with heat-killed *B. pertussis* [98]. Following challenge infection with *B. pertussis*, clinical symptoms, pathological alterations, and bacterial shedding were significantly reduced in piglets that had received passively transferred immunity (P≤0.004). It should be noted that in pigs, maternal antibodies are transferred after birth through colostrum [99].

Human studies

Most studies involving vaccination of pregnant women were carried out in the 1930s to 1950s (Table 4). Initially, the aim of the studies was to determine whether protective antibodies were transferred from mother to child. Protective antibodies were assayed in vitro by opsono-
phagocytosis or in vivo by their ability to protect mice from a lethal challenge [54]. Pregnant women selected for these studies had not been vaccinated previously, although most probably had contracted pertussis. In general, high vaccine doses were used, up to six injections each containing $10^{10}$ to $50 \times 10^{9}$ bacteria (a single dose of a current whole-cell vaccine contains approximately $16 \times 10^{9}$ bacteria). Injections were given in the arm, subcutaneously, or intramuscularly, in the third trimester.

In all studies, levels of B. pertussis-specific antibodies increased in the newborn baby after maternal vaccination compared with newborn babies from untreated mothers. The systemic reactions observed in the mother were few and not severe according to the authors. Local reactions were common, at times very painful, not serious, and sometimes lasted as long as a few days. No effect on the pregnancy or delivery.

In one study, protection in the child was assessed [100]. In this study, the incidence of pertussis in a group of 100 babies of immunized mothers was compared with an equal number of babies of unimmunized mothers. In the first 6 months of infancy, there were six exposures in the unimmunized group, resulting in three cases of pertussis. During the same period there were eight exposures in the immunized group, but no cases of pertussis developed. In the second 6

<p>| Table 4. Maternal vaccination of pregnant women with pertussis whole-cell vaccines. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Number of pregnant women</th>
<th>Number of injections</th>
<th>Total dose of bacteria</th>
<th>Side-effects in neonates</th>
<th>Side-effects in pregnant mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lichty et al. (1938) [129]</td>
<td>42</td>
<td>Three</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Mishulow et al. (1942) [54]</td>
<td>29</td>
<td>Three</td>
<td>$90 \times 10^9$ to $150 \times 10^9$</td>
<td>NR</td>
</tr>
<tr>
<td>Cohen and Scallon (1943) [130]</td>
<td>167</td>
<td>Six</td>
<td>$80 \times 10^9$, $150 \times 10^9$</td>
<td>No premature births or postpartum complications which could be attributed to the inoculations.</td>
</tr>
<tr>
<td>Kendrick et al. (1945) [53]</td>
<td>57</td>
<td>Three</td>
<td>$25 \times 10^9$</td>
<td>NR</td>
</tr>
<tr>
<td>Cohen and Scallon (1946) [100]</td>
<td>170</td>
<td>NR</td>
<td>$90 \times 10^9$, $150 \times 10^9$</td>
<td>No fever and systemic reactions. Local discomfort. No ill effects upon the pregnancy.</td>
</tr>
<tr>
<td>Adams et al. (1947) [131]</td>
<td>16</td>
<td>Three</td>
<td>$190 \times 10^9$</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR = not reported. Vaccinations in all studies took place in the third trimester of pregnancy.
months of life each group yielded two cases of pertussis. The latter finding suggests that the passive immunity conferred to the babies did not persist after 5-6 months of age. Side-effects in the mother, as observed in these studies (Table 4), would not be acceptable today. However, whole-cell vaccines have now been replaced by less reactogenic acellular vaccines. Acellular vaccines have been tested in adults in a number of studies (see below) and no serious side-effects were observed.

**Safety record of maternal vaccination**

Maternal vaccination against tetanus has been used worldwide since the 1970s [101] and no evidence has been presented of negative effects on pregnancy or the neonate. Vaccination of pregnant women has been practiced extensively in the USA since 1957 [29, 102]. The greatest use occurred before 1966, when vaccination with both influenza and poliovirus vaccines was recommended during pregnancy. The safety of this procedure was documented by the large Collaborative Perinatal Project in which over 50,000 pregnant women were enrolled between 1959 and 1965 [103]. The offspring was followed until 7 years of age for malformations, hearing impairment, and learning disabilities. The most common vaccine used was inactivated poliovirus vaccine, which 18,342 women received, whereas 3,056 received live attenuated oral poliovirus vaccine at some time during pregnancy. Influenza vaccine was given to 2,291 women. Based on extrapolation from the vaccination rates of women in this study, it is estimated that 2 million doses of vaccines were given to pregnant women each year between 1959 and 1965. Vaccination of women during pregnancy was not associated with adverse outcomes or an increased risk for any disability in the infants in this study. However, these studies might not have been large enough to detect congenital abnormality or foetal loss. Inactivated influenza vaccine is currently recommended by the US Centers for Disease Control and Prevention for all pregnant women with high risk factors for severe influenza and for those women who will be in the second or third trimester during the influenza season [104].

Controlled trials done in recent years with bacterial (Hib, group B streptococci, *S. pneumoniae*) and viral vaccines (influenza, respiratory syncytial virus, rubella virus, poliovirus) have provided no evidence of adverse outcomes for mother or child [28, 105-109]. Furthermore, no evidence was found that maternal vaccination affected active vaccination of infants. Infants of women who received vaccines had, in general, developed responses to active vaccination that were similar to those of infants whose mothers were not vaccinated [29].
Safety of acellular vaccines in adults

Maternal vaccinations have been carried out with whole-cell vaccines, which are known to be relatively reactogenic. In many countries, whole-cell vaccines have been replaced by acellular vaccines, which show fewer side-effects compared with whole-cell vaccines [110]. To reduce circulation of *B. pertussis* in human populations, universal adolescent and adult vaccination is considered, or already implemented, in a number of countries [7, 111].

A large proportion of adults may have high pertussis antibody levels because of recent infection with *B. pertussis* [112] and there is some concern that using infant doses for adult vaccination could result in side-effects because of high levels of pre-existing antibodies. Therefore, adult formulations have been developed that have a lower content of antigens compared with infant pertussis vaccines. Safety trials have been done with vaccines with adult formulations. In most studies a single booster was given. However, in one trial a second dose of acellular vaccine was given [113]. The second dose was not associated with increased adverse events in adults but elicited increased antibody titres over that achieved by a single dose against pertussis toxin only. In another study [114], it was noted that vigorous serum antibody responses to several pertussis antigens were associated with an increased risk of developing late-onset reactions (pain, tenderness, induration or erythema at the site of injection), as has been observed after vaccination with diphtheria toxoid [115]. If high levels of pre-existing immunity are a risk factor for more serious side-effects, it may be advisable to limit vaccination of pregnant mothers to those with low anti-pertussis titres.

The studies with acellular vaccines in adolescents and adults showed that side-effects were generally mild and not substantially different from control vaccines without the pertussis component (e.g., vaccines containing diphtheria and tetanus toxoids) [116]. Furthermore, the adult formulations were immunogenic and, importantly, pre-existing antibody did not have an inhibitory effect on the response to vaccination [116, 117]. It seems likely that acellular vaccines will have a much improved safety profile in pregnant women compared with the whole-cell vaccines that have been tested. In fact, according to the recommendations of the US Advisory Committee on Immunization Practices (ACIP), pregnancy is not a contraindication for vaccination with adult formulations of a combined tetanus, diphtheria, acellular pertussis vaccine [118].

Discussion

Maternal vaccination may substantially reduce pertussis morbidity and mortality in infants too young to be fully vaccinated. A major obstacle for its introduction is concern about side-effects in mother and child. However, maternal vaccination has been shown to be effective and
safe for tetanus toxoid over long periods of time. Furthermore, maternal vaccination studies with whole-cell vaccines done in the past have not shown serious side-effects in mother or child. In one study, maternal vaccination was shown to protect newborn babies. Because maternal vaccination transfers immunity to the infant through antibodies, it is significant that studies in animals and human beings have shown that antibodies confer protection against pertussis, albeit in varying degrees. An important gap in our knowledge is the level of maternal antibodies required for protection of the child. However, levels of antibody against pertussis toxin, pertactin, and fimbriae (or agglutinogens) have been established that are associated with protection, and adult formulations of acellular vaccines induce high levels of antibodies against these antigens. Importantly, adult formulations of acellular vaccines have been found to be safe and are recommended for adolescent and adult vaccination in a number of countries. Additionally, pregnancy is not deemed a contraindication for vaccination with adult formulations of acellular vaccines according to the recommendations of the ACIP. However, there is some evidence that high levels of pre-existing immunity in adults are a risk factor for side-effects of vaccination and this issue needs to be investigated further. Another issue that should be addressed is whether maternal immunization will affect the infant’s immune response to primary vaccination. Ultimately, safety and efficacy of maternal vaccination will have to be assessed in clinical trials. An advantage of maternal vaccination is the accessibility of the targeted group, since both (pregnant) mothers and infants frequently visit health-care centres. Finally, maternal vaccination could also have beneficial effects for childhood diseases other than pertussis, especially in the low-income countries where it could help to prevent the 2-3 million neonatal and early infant deaths every year [119].

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Main findings

The aim of the Dutch National Immunization Programme is to protect the population and society against severe infectious diseases by means of vaccination [1]. Despite more than 50 years of programmatic vaccination with a high coverage (circa 96%), pertussis is still endemic in the Netherlands. Since 1996, the reported number of patients varied every year between 3,000 (i.e., 18 per 100,000 persons) and 10,000 (i.e., 60 per 100,000 persons) and these figures represent the ‘tip of the iceberg’ as the majority of infections go unrecognized. Each year, approximately 200 patients - predominately young unvaccinated infants - are hospitalised and on average one death due to pertussis is reported [2]. The preschool booster vaccination, introduced in 2001, caused a significant decline in the incidence of pertussis among the targeted population and resulted in a reduction in infant morbidity (chapter 2). The shift to an acellular vaccine in 2005 has decreased morbidity in 1-2-year-olds and resulted in a reduction of the number of side effects due to vaccination [3, 4]. However, the number of infections in adults more than doubled in the past decade (chapters 2 and 6). Although the majority of adult infections are mild or subclinical, the high circulation of B. pertussis infections poses a significant risk of transmission to unprotected newborns in whom infections often lead to severe disease and possibly long term sequelae (chapters 4 and 8).

Understanding the disease dynamics and control of pertussis is challenging experts in the field of epidemiology, microbiology, mathematical modelling, immunology and medicine. Studying pertussis disease dynamics is complicated by the lack of specific symptoms in most of the infected persons, especially in adolescents and adults (chapters 6 and 8), making pertussis diagnosis problematic. Control of pertussis is hampered by the fact that there is no accurately defined correlate of protection [5, 6].

The studies presented in this thesis were designed to provide insight in the epidemiology of pertussis in the Netherlands in the past decade and to identify ways to optimize protection of the population, in particular infants, against pertussis. Based on our studies, I will first discuss what I believe are the most likely causes of the dramatic increase in pertussis incidence. Finally, I will propose a number of measures to improve the control of pertussis in the Netherlands.

Explanations for the trends in epidemiology

Increased awareness and improved diagnostics

Since morbidity [7-9] and mortality [10] are generally underestimated in routine surveillance systems, increased awareness leads to a larger part of patients being identified and/or reported. The large variation in reported incidence of pertussis in Europe (e.g., in 2007 the reported...
incidence was 115/100,000 in Norway; 44/100,000 in the Netherlands; and 1.7/100,000 in Denmark) rather reflects differences in awareness, reporting and diagnosis than only differences in vaccination history and population immunity [11]. In the Netherlands, increased attention to pertussis in (medical) journals after the epidemic in 1996 and subsequent changes in the vaccination schedule, will have increased the index of suspicion among clinicians for pertussis in patients with prolonged cough. Interestingly, a dramatic increase of the reported incidence of clinical pertussis cases has also been observed in many other countries, despite a high vaccination coverage [5, 12-15]. Since it is unlikely that awareness improved in a similar way at the same time in different countries, better awareness alone cannot explain the simultaneous resurgence of the disease.

Improved diagnostics is often put forward in literature as explanation for the resurgence of pertussis. In the Netherlands, serological tests for pertussis were exclusively performed at the RIVM before 1996, but since 1998 other laboratories have started to perform serology with commercial available assays. This decentralization may have facilitated pertussis diagnosis resulting in more reliable (and higher) notification rates. The impact of a more wide-spread use of PCR as a diagnostic method is negligible, as only 5% of notified patients is confirmed by PCR each year.

Although the above mentioned changes will have contributed to the observed increase in reported pertussis, the significant increase in the seroprevalence (chapter 6) especially among adults, indicates the true number of infections has also increased. Consistent with this, incidence rates of hospitalisations - which are less affected by changes in clinical practice and diagnostic methods – have also increased among adolescents and adults (chapter 2).

Suboptimal vaccines, waning immunity and pathogen adaptation

I propose the increase in the number of infections in the last decade may be the compound effect of suboptimal vaccines, waning immunity and pathogen adaptation.

Estimations of vaccine effectiveness in 1-year-olds show that the VE was above 90% in 1989-1993, and decreased to 30% in 1996-1998 [16]. The use of a suboptimal vaccine [17], is likely to have resulted in a cohort of susceptible children and an increased circulation of the causative pathogen. This is illustrated by a shift of the peak incidence from infants towards preschool children and an increase in incidence over a broad age-range in 1996.

Concurrently, changes in circulating \textit{B. pertussis} strains were observed. Variations of genes coding for pertactin and pertussis toxin were demonstrated that differed from the vaccine type strains [18] and this mismatch affected vaccine efficacy in a mouse model [19-21]. Besides, a change has been observed with respect to the promoter for Ptx (ptxP) which is a major
virulence factor and component of all pertussis vaccines [22]. The majority of strains isolated since 1996 (ptxP3 strains) appear to produce more pertussis toxin. Pertussis toxin suppresses the immune response and thus an increased production may enhance virulence [22-24]. The concurrence in time between the emergence of ptxP3 strains and the shift of disease towards older age-categories, suggests a causal relationship (Figure 1). By increasing suppression of host immune defences, these more virulent strains may be able to infect at higher levels of host immunity, decreasing the duration of vaccine-induced immunity. This assumption is supported by the increased seroprevalence and a growing number of symptomatic infections in adults (this thesis). Apparently, B. pertussis has adapted to hosts with waning immunity in order to maintain a bacterial reservoir in a population with high vaccination coverage.

![Figure 1. Incidence of notifications and hospitalisations for pertussis per 100,000 persons per year, and the prevalence of ptxP3 strains in the Netherlands, 1989-2008.](image)

**Importance of integration of surveillance data**

As has been clearly demonstrated for pertussis, understanding the epidemiology of vaccine-preventable diseases requires the systematic collection and integration of data on clinical cases, seroprevalence, and strain variation. The combination of notifications and seroprevalence is required to give insight in underreporting and the (changes in) severity of infections. Although the true number of pertussis infections is more than 100 times higher than the reported number of infections (chapter 6), underreporting does not invalidate monitoring the impact of vaccination by trend analysis of the number of clinical cases. Studying clinical surveillance data has increased our knowledge on pertussis and
has directly contributed to the implementation and evaluation (chapter 2) of control measures such as the preschool booster [17]. The notification system is currently the only surveillance system available to monitor trends in the incidence according to the vaccination status of cases. Given the changes in the vaccination programme in recent years and the upward trend in pertussis notifications (chapter 6), the current system of notifications should therefore be continued. Surveillance of hospitalisations registered by the National Medical Register is also of utmost importance as hospitalisations are less subject to surveillance artefacts and therefore allows better comparison of pertussis disease burden between countries.

In chapter 6, we showed that significant changes in the age-specific prevalence of high anti-Ptx IgG levels in a ten year period, agree with trends observed in reported incidence of pertussis at the time. To monitor trends in circulation of the pathogen in the short term, opportunistically collected samples (e.g., from blood donors or residual sera) could be tested for the concentration IgG-Ptx. Ideally, to gain insight in the occurrence of (mild) infections and possibly to find a correlate of protection, a random cohort (preferably also followed for other study purposes) of persons stratified by age and duration since last vaccination could be requested to keep a diary on pertussis related symptoms and coughing contacts, and to give blood every three months in order to assess their concentration pertussis related antibodies (i.e., antibodies against Ptx, Prn, Fim, FHA).

Strain surveillance is required to assess whether increases in notification are due to changes in vaccine quality or changes in the pathogen population [25]. Further, by studying the nature of the changes in the pathogen population, interventions can be proposed [26]. Since culture has almost completely been replaced by serology and PCR as method of laboratory confirmation, it is highly recommended that a (sentinel) system is set up that allows the systematic collection of Bordetella strains, preferably not only from the Netherlands but also from abroad. Since acellular vaccines confer no protection against *B. parapertussis* infections [27-30] - which can cause similar symptoms as *B. pertussis* [31, 32] - the occurrence of *B. parapertussis* infections should also be monitored.

**Implications of the increased circulation for public health management**

In chapter 8, we showed that only 9% of infected household members was treated with antibiotics before onset of illness in the infant and only one of the 164 investigated families received prophylactic treatment conform the recommendations [33, 34]. This indicates that many general practitioners (GPs) do not recognize pertussis in adolescents and adults or do not acknowledge the potential severity of infection in young infants. Besides typical pertussis symptoms, many adult patients present to their GP with lack of sleep due to
paroxysmal coughing, sweating attacks and cold symptoms [9]. General practitioners should actively ask patients with persistent cough for minimal one week in combination with one of the above symptoms, whether he or she has contact with infants or pregnant women. If the latter is true and suspicion for pertussis is high, for instance as multiple cases in one practice are found, promptly start of antibiotic treatment should be considered [34]. Early diagnosis and treatment of pertussis can prevent severe pertussis in infants and limits the spread of the bacterium. Furthermore, it prevents unnecessary medical procedures, such as bronchoscopies, CT scans, or allergy testing, prompted for suspicion of other diseases [35, 36]. As only a fraction of adults with presumptive pertussis infection visits a general practitioner, public campaigns that inform on infection control measures, such as cover-your-cough campaigns, will help to reduce exposure and transmission [37].

Implications for pertussis vaccination policy
Vaccination has had a tremendous impact on the incidence of pertussis. Indeed, after abandoning of pertussis vaccination, incidence rates of reported pertussis in Sweden increased to 3,370 per 100,000 and in Japan an epidemic occurred in 1979 with more than 41 deaths [38, 39]. Conversely, immediately after re-introduction of vaccination in Sweden and Japan, the incidence of pertussis among vaccinated age groups decreased to similar levels as before the period of vaccine withdrawal [39, 40]. The aim of vaccination can be eradication, elimination, or containment of a disease [41]. Eradication is only feasible for diseases where vaccination offers lifelong protection and high vaccination coverage is achieved. Elimination involves achieving a level of immunity within a population, such that an infectious disease has very little scope to proliferate but the pathogen is still present in the population. Containment can be defined as the point at which the disease, although not eliminated, is no longer a significant health problem. With the current pertussis vaccines that induce only temporarily protection against disease severity and transmission, containment or minimization of disease burden will be the maximum achievable. There is however no level defined when containment is reached. The preschool booster and the replacement of the Dutch whole-cell vaccine by an acellular vaccine have successfully reduced the disease burden in children. However, the switch to acellular vaccines will not affect morbidity in infants too young to be vaccinated. In Canada the switch to acellular vaccines was made already in 1997, but this has had little influence on the incidence in infants below 3 months of age [42]. Moreover, vaccination with acellular vaccines in childhood does not prevent (re-)infection in adulthood as vaccine induced immunity wanes within 6-10 years [43]. Within households, vaccine induced immunity may even wane more quickly: a third of the children in the BINKI-study (chapter 8) who were vaccinated with the
acellular vaccine were again infected within 4 years after completion of the primary series.
The ultimate goal of pertussis vaccination should be to eliminate severe disease and death
among infants and young children. Therefore, the increased circulation of pertussis (chapter 6),
demands for additional intervention measures to prevent severe pertussis in young infants.

Vaccination measures for the short term
Booster vaccinations for adults will reduce the circulation of pertussis and thus transmission to
infants. However, adult booster vaccination may not be the most effective strategy to control
pertussis. First of all, due to waning immunity repetitive boosting will be required with the
current vaccines [44]. Not surprisingly, many published papers in favour of adolescent and
adult vaccination are written by authors linked to the pharmaceutical industry. Secondly,
repeatedly vaccinating adults is a very labour-intensive and expensive matter and will not
likely be cost-effective (chapter 3). In Canada, it took five years to implement a booster
programme for adolescents and adults and implementation occurred only after organizing
comprehensive educational programmes to convince authorities, health care providers, and
the public of the need of such a programme [45]. Thirdly, personal risk perception may be
too low in adolescents and adults to consider pertussis immunization [46]. In chapters 6 and
8, we showed that the majority of infections in these groups are relatively mild. Besides,
the recently conducted vaccination campaign for HPV illustrated that besides risk perception
many other factors determine willingness to vaccinate beyond childhood [47]. Finally, adult
infections have always occurred [48], and since disease is relatively mild compared to disease
in infants and children it is debatable whether infections in adults are experienced as a public
health problem and justify the public investment of general adult booster vaccinations. When
transmission remains high, mild infections among adults will be frequent and will boost
clinical immunity, making revaccination redundant. To make appropriate decisions about the
vaccination of particular target groups, seven criteria have been formulated by the Health
Council [49] grouped under five thematic headings: seriousness and extent of the disease
burden, effectiveness and safety of the vaccination, acceptability of the vaccination, efficiency
of the vaccination, and priority of the vaccination. It is doubtful whether adult vaccination
satisfies these criteria.

Instead of general adults booster vaccinations it will be more (cost-)effective to give booster
vaccinations only to adults who are in close contact with infants (such as young parents and
health care workers taking care of infants) to reduce transmission. Moreover, feasibility of this
‘cocooning strategy’ will be better as young parents are motivated to protect their baby and
have frequent contact with health care.
**Vaccination measures for the long term**

Vaccinating directly after birth or giving the first dose at six weeks of age, may confer earlier protection against pertussis [50, 51]. Research is needed, however, to demonstrate that early induced immunity does not interfere with subsequent vaccinations or results in an impaired immune response against other vaccine components [52, 53]. Ideally, vaccination of mothers during pregnancy is the best option to protect infants from birth until immunity is induced by active vaccination (chapter 9). However, concerns for safety make this strategy almost unrealizable for a disease like pertussis.

Although each of these approaches may be effective, the most (cost-)effective way to reduce the pertussis burden is to introduce vaccines which induce long lasting immunity. The vaccines could be improved so that their composition reflects the antigenic make-up of circulating strains and, in particular, they induce Ptx neutralizing antibodies that persist [22].

**General conclusion**

The studies in this thesis have shown that despite changes in the vaccination programme which successfully reduced morbidity in childhood, the circulation of *Bordetella pertussis* has increased especially in the adult population. In fact, vaccination in childhood may have led to the evolution of more virulent strains and a shift of the reservoir of *B. pertussis* to adults.

However, since the disease burden in the adult population seems relatively confined, the main reason for introducing new vaccination strategies should be to prevent severe pertussis in infants who are too young to be protected by vaccination.

Due to integration of clinical, strain, and immune surveillance data, the Dutch pertussis surveillance system is one of the most comprehensive systems, and surveillance and forthcoming studies are conducted without conflict of interest. Based on the results of this surveillance we propose the following recommendations:

- Young parents, and people who have close contact with young infants (such as health care workers taking care of infants) should receive booster vaccinations.
- To justify an eventual introduction of general adult booster vaccinations, the burden of disease in adults in the Netherlands should be determined.
- The potential long term effects of severe pertussis, and of infant pertussis in general, on loss of quality of life should be further investigated.
- Governmental policy makers and critical researches on pertussis should incite vaccine manufactures to invest in new pertussis vaccines that induce long lasting immunity.
At any point in time, the immunity of the population is the compound effect of disease and vaccination experience and thus the recommendations in this thesis are given for the current situation. Since history of vaccination, the vaccines used, the schedule vaccination and the coverage vary from country to country; care should be taken to generalize these recommendations to other countries. In fact, for many developing countries the challenge is simple to achieve high vaccination coverage of timely immunisation for infants [54]. However, the findings generated from the Dutch surveillance may inspire researchers in countries, and hence will contribute to a better control of the burden of pertussis worldwide.

References

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Summary

Pertussis is a highly contagious infectious disease of the respiratory tract, which can cause paroxysmal coughing lasting for more than a month, often associated with inspiratory whooping and/or post-tussive vomiting. Before childhood vaccination against pertussis was introduced in the 1950s, pertussis was a major cause of infant death, with 350 deaths per year in the Netherlands. Vaccination successfully decreased the morbidity and mortality due to the disease. However, in 1996 a resurgence of pertussis was observed in the Netherlands despite more than 40 years of programmatic vaccination with a high coverage (chapter 1). Since then, the incidence of pertussis has remained high with between 3,000 and 10,000 reported patients annually and around 200 hospitalisations predominately involving young children. Still, these figures represent the ‘tip of the iceberg’ as the majority of infections are mild and often go unrecognized, especially in adolescents and adults. In infants, however, infection is most severe and complications may develop, such as pneumonia, encephalopathy or even death.

Routine vaccination against pertussis was introduced in the Dutch National Immunization Programme in the 1950s with a combined diphtheria, pertussis, tetanus and polio (DTP-IPV) vaccine at 3, 4, 5, and 11 months. Due to the re-emergence of pertussis several changes in vaccination strategy were implemented in the last decade: in 1999 the vaccination schedule was accelerated and the first dose of pertussis vaccine is now given at 2 months instead of 3 months of age, in 2001 a preschool booster was introduced for four-year-olds, and in 2005 the Dutch whole-cell vaccine was replaced by an acellular vaccine. Whole-cell vaccines are based on killed and detoxified bacteria, whereas acellular vaccines consist of several purified antigens of the causative organism *Bordetella pertussis*.

The National Institute for Public Health and the Environment (RIVM) monitors and investigates the effectiveness, safety and reliability of (changes in) the National Immunization Programme by means of surveillance. Trends in the occurrence of pertussis are studied by means of disease surveillance and immunosurveillance. Since widespread vaccination may force pathogens to adapt to vaccine-induced immunity, trends in the epidemiology are interpreted in relation to changes observed in phenotypic or genotypic characteristics of *B. pertussis*.

The main objectives of the studies described in this thesis are to explain trends in the epidemiology of pertussis in the Netherlands in the past decade and to guide policy and development of control strategies for pertussis. Results from studies on the disease burden (chapters 2-4), infection frequency (chapters 5-6), and transmission routes (chapters 7-8) for pertussis are used to recommend optimal vaccination strategies (chapters 9-10) to decrease the burden of pertussis in the Netherlands.
**Burden of pertussis**

In chapter 2, we determined the impact of the preschool booster for four-year-olds on the burden of pertussis. We showed that the incidence of hospitalised and reported pertussis cases significantly decreased among the targeted population. Another important finding of our study was the decreasing trend in the incidence of pertussis in infants, suggesting that transmission from siblings to susceptible infants was reduced after introduction of the preschool booster. In contrast to the reduction in infants, in the same period the incidence of reported cases among cohorts aged 10-19, 20-59, and ≥60 years, increased with 60%, 44%, and 68%, respectively. Despite the substantial reduction in the number of cases in childhood, the preschool booster was not considered cost-effective (chapter 3). By studying age-specific health care utilization and costs associated with pertussis, we found that the economic burden of pertussis is largely determined by costs per infant case (€1,491) and only to a limited degree by costs per patient in other age-groups (circa €75). Thus, costs of pertussis in adolescents and adults are relatively limited, and prevention of pertussis in infants will be the most effective way to save expenses. More importantly, from a public health point of view, young infants suffer from the most severe form of disease. Besides, severe pertussis in infancy may have consequences on the long term. In chapter 4, we studied whether there is an association between pertussis in infancy and respiratory and cognitive disorders in early childhood. We compared a group of 89 children aged 13-45 months and hospitalised for laboratory confirmed pertussis within the first six months of their life, with 172 age-matched children without a history of pertussis. Children with a history of pertussis more often showed “asthma like symptoms” on toddler age and were more likely to report “respiratory infections”. Although it is unclear whether there is a causal relation between pertussis infection in infancy and respiratory illness in childhood, this deserves further attention.

From chapters 2-4 we conclude that while vaccination has successfully reduced the pertussis disease burden in childhood, the number of pertussis cases in adults is growing. The latter increases the infection pressure in infants and thus will have a negative impact on the efficacy of childhood vaccination. This emphasizes the need for further measures to prevent severe pertussis in young infants, either by interrupting common routes of transmission or by improving infant immunity to pertussis.

**Infection frequency**

Surveillance artifacts such as increased awareness and improved diagnostics may have contributed to the observed increase of reported pertussis. The frequency of pertussis infection is most reliably estimated on the basis of the prevalence of high IgG concentrations against pertussis toxin (IgG-Ptx), which is not affected by these surveillance artifacts.
Since mothers suffering from pertussis at the time of delivery have a high chance of infecting susceptible newborns, in chapter 5 we determined the prevalence of infection in pregnant women. In total, 20 (6.3%) of 315 pregnant women in our study showed serological evidence of *B. pertussis* infection during or shortly before pregnancy, which is much higher than the incidence of reported cases in this age group as shown in chapter 2. To study the age-specific infection frequency in the general population, a cross-sectional population-based serosurveillance study was conducted in 2006-07 (chapter 6). For 7,903 participants serum antibody concentrations were analysed and the age-specific seroprevalence (0-79 years) was compared with the seroprevalence obtained from a similar national survey conducted in 1995-96 and with incidence rates calculated from mandatory notifications in both periods. In 2006-07, 9.3% of the population in the Netherlands above 9 years of age had a pertussis infection in the past year, indicating at least a twofold increase compared to the 1995-96 survey (4.0%). Interestingly, the seroprevalence followed a similar trend as the reported incidence between both periods. Obviously, trends in notifications reliably reflect changes in the circulation of pertussis.

*Transmission routes*

To gain insight in pertussis disease dynamics and common routes of transmission, we studied the age-specific long-term periodicity and seasonality of pertussis in the Netherlands (chapter 7). The concurrent annual fluctuation of pertussis incidence that we found in adults and young children, suggests frequent transmission within and between these age groups. This finding was confirmed by the results of a household transmission study named BINKI (which is an acronym in Dutch for: Baby’s geINFecteerde met Kinkhoeest) in which we aimed to identify the most likely sources of pertussis infection in young infants (chapter 8). A total of 560 household contacts of 164 infants hospitalized for pertussis were examined by polymerase chain reaction, culture, and serological testing to establish *B. pertussis* infection. Of the household contacts, 53% had laboratory-confirmed pertussis. The most likely source of infection in the infant was a sibling (41%), mother (38%), or father (17%). These data show that selective vaccination of people in close contact with infants (cocooning strategy) will likely reduce transmission of pertussis to infants. Since expectant parents have regular contact with health care and will be well motivated to protect their child, cocooning seems a feasible strategy and its implementation needs urgent consideration on the short term.

*Future vaccination strategies*

Current vaccines only induce transient protection against pertussis and after a few years the risk of (transmission of) infection recurs. Therefore, in chapter 9, we discuss the possibilities
of maternal vaccination in order to directly improve immunity of infants against pertussis. By placental transfer of maternal antibodies, vaccination in the third trimester of pregnancy may confer protection from the moment of birth. However, due to ethical, technical, and legal dilemmas it is problematic to investigate the efficacy and safety of this strategy in clinical trials.

Finally, in the general discussion of this thesis (chapter 10), we discuss the most likely causes of the dramatic increase in pertussis incidence in the past decade and propose measures to improve the control of pertussis.

In literature, different reasons have been given for the cause of the increase in pertussis in the last decade: increased awareness, improved diagnostics, suboptimal vaccines, waning vaccine-induced immunity and pathogen adaptation. Based on the integration of clinical-, pathogen- and immunosurveillance data we argue that the increase of pertussis in the Netherlands cannot solely be attributed to increased awareness and improved diagnostics. Indeed, the increase in the seroprevalence demonstrates that the circulation of the causative pathogen has also increased. The concurrence in time between the emergence of new pertussis strains and the increase in seroprevalence among adults suggests the pathogen has adapted to hosts with waning immunity resulting in a large bacterial reservoir.

Despite the high prevalence of pertussis in adolescents and adults, pertussis in these groups is relatively mild compared to disease in infants and children. Thus, the aim of pertussis vaccination should be to eliminate severe disease and death among infants and young children. The preschool booster and the replacement of the Dutch whole-cell vaccine by an acellular vaccine have successfully reduced the disease burden in children. However, the increased circulation of pertussis in adults calls for additional interventions such as cocooning to prevent transmission to young infants. Ultimately, vaccines that induce long lasting protection against pertussis should be developed.
Samenvatting
Samenvatting

Kinkhoest is een zeer besmettelijke respiratoire infectie veroorzaakt door *Bordetella pertussis* en in mindere mate door *Bordetella parapertussis*. De ziekte kenmerkt zich door hoestaanvallen die gedurende meer dan een maand kunnen optreden. De hoestaanvallen worden vaak gevolgd door braken en kunnen zo heftig zijn dat ze leiden tot ademnood, bloeduitstortingen en botbreuken. Bij jonge ongevaccineerde baby’s worden ernstige symptomen en complicaties waargenomen, zoals pneumonie en encefalopathie. In sommige gevallen leidt kinkhoest zelfs tot sterfte. Voordat vaccinatie halverwege de vorige eeuw werd ingevoerd, was kinkhoest een belangrijke oorzaak van sterfte bij kinderen, met circa 350 sterfgevallen per jaar in Nederland. Door vaccinatie is de morbiditeit en mortaliteit door kinkhoest sterk afgenomen. Ondanks een continu hoge vaccinatiegraad werd er in 1996 een epidemie van kinkhoest waargenomen (hoofdstuk 1). In de jaren na 1996 is de incidentie van kinkhoest hoog gebleven. Jaarlijks worden 3.000 tot 10.000 kinkhoestpatiënten door de GGD gemeld aan het Centrum voor Infectieziektebestrijding in het kader van de meldingsplicht. Van deze patiënten worden er ongeveer 200 opgenomen in het ziekenhuis, voornamelijk kinderen jonger dan 3 maanden. Toch vormen deze aantallen slechts ‘het topje van de ijsberg’, aangezien het grootste deel van de infecties – vooral bij tieners en volwassenen - mild of asymptomatisch verloopt.

Sinds 1957 is vaccinatie tegen kinkhoest in een combinatievaccin met difterie, kinkhoest, tetanus en polio (DKTP) opgenomen in het Rijksvaccinatieprogramma (RVP). Door de sterke toename van kinkhoest zijn er in de afgelopen 10 jaar enkele veranderingen ten aanzien van de kinkhoestvaccinatie doorgevoerd in het RVP. In 1999 is het vaccinatieschema vervroegd en sindsdien wordt niet meer gevaccineerd op de leeftijd van 3, 4, 5 en 11 maanden, maar op de leeftijd van 2, 3, 4 en 11 maanden. Vanaf oktober 2001 krijgen vierjarigen een voorschoolse boostervaccinatie met een acellulair vaccin en sinds 2005 wordt ook voor de vaccinaties in het eerste levensjaar een acellulair vaccin gebruikt in plaats van het hele-cel vaccin. Hele-cel vaccins bestaan uit gedode hele bacteriën, terwijl acellulaire vaccins een aantal gezuiverde oppervlakte-eiwitten van de verwekker *B. pertussis* bevatten.

Het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) evalueert en onderzoekt de effectiviteit, veiligheid en gevolgen van (veranderingen in) het Rijksvaccinatieprogramma door middel van surveillance. Trends in het voorkomen van kinkhoest worden bestudeerd met behulp van klinische- en immuunsurveillance. Ziekteverwekkers, zoals de bacterie *B. pertussis*, kunnen onder druk van vaccinatie veranderen waardoor bacteriestammen kunnen ontstaan waartegen het vaccin minder goed beschermt. Daarom worden trends in het voorkomen van kinkhoest bestudeerd in relatie tot fenotypische en genotypische veranderingen in *B. pertussis*.
De studies in dit proefschrift hebben tot doel de trends in de epidemiologie van kinkhoest in Nederland in de afgelopen tien jaar te verklaren en het beleid ten aanzien van kinkhoestvaccinatie te adviseren over maatregelen voor de bestrijding van kinkhoest. Resultaten van studies over de ziektelast (hoofdstukken 2-4), infectiefrequentie (hoofdstukken 5-6) en transmissieroutes (hoofdstukken 7-8) van kinkhoest worden gebruikt om aanbevelingen te doen over optimale vaccinatiestrategieën (hoofdstukken 9-10) om de ziektelast door kinkhoest in Nederland te verlagen.

Ziektelast
In hoofdstuk 2 beschrijven we de resultaten van het onderzoek naar het effect van de voorschoolse boostervaccinatie op de ziektelast door kinkhoest. We toonden aan dat de incidentie van ziekenhuisopnamen en wettelijke meldingen wegens kinkhoest aanzienlijk is afgenomen in de leeftijdsgroepen die deze vaccinatie hebben gekregen. Een andere belangrijke bevinding was de afname in de incidentie van kinkhoest bij baby’s. Dit doet vermoeden dat na de invoering van de voorschoolse booster de transmissie van broertjes en zusjes naar vatbare baby’s is verminderd. In tegenstelling tot de dalende trend bij baby’s, is in dezelfde periode de incidentie van het aantal gemelde patiënten in de leeftijdsgroepen van 10-19, 20-59 en ≥60 jaar met respectievelijk 60%, 44% en 68% toegenomen.

Ondanks de aanzienlijke vermindering van het aantal kinkhoestgevallen bij kinderen, is de voorschoolse booster waarschijnlijk niet kosteneffectief (hoofdstuk 3). In een studie naar het zorggebruik en de kosten die gepaard gaan met kinkhoest, vonden we dat de economische last van kinkhoest grotendeels wordt bepaald door baby’s met kinkhoest (€1490 per patiënt) en slechts in beperkte mate door de kosten voor kinkhoestgevallen in andere leeftijdsgroepen (circa €75 per patiënt). De economische last van kinkhoest bij tiener en volwassenen is dus relatief klein en preventie van kinkhoest bij baby’s zal de meest effectieve manier zijn om kosten te besparen. Ook vanuit het oogpunt van de volksgezondheid is preventie van kinkhoest bij pasgeborenen opportuun, aangezien in deze groep de ziekte vaak het meest ernstige verloop heeft. Daarnaast kan ernstige kinkhoest op jonge leeftijd gevolgen hebben voor de ontwikkeling van het kind op de lange termijn.

In hoofdstuk 4 beschrijven we de resultaten van een onderzoek naar het mogelijke verband tussen het doormaken van kinkhoest op jonge leeftijd en respiratoire aandoeningen en de cognitieve ontwikkeling op peuter- en kleuterleeftijd. Hiertoe hebben we een groep van 89 kinderen in de leeftijd van 13-45 maanden, die in hun eerste levenshalfjaar in het ziekenhuis zijn opgenomen vanwege kinkhoest, vergeleken met 172 naar leeftijd gematchte kinderen zonder een voorgeschiedenis van kinkhoest. Kinderen, die in de eerste 6 maanden van hun leven kinkhoest hadden doorgemaakt, rapporteerden vaker “astma-achtige symptomen” op
peuterleeftijd en bleken vaker “infecties van de luchtwegen” te hebben doorgemaakt. Hoewel het onduidelijk is of er een causaal verband is tussen het doornemen van een kinkhoestinfectie op jonge leeftijd en het voorkomen van respiratoire aandoeningen in de kindertijd, verdient de mogelijke relatie verder onderzoek.

Op basis van hoofdstuk 2-4, concludeerden we dat vaccinatie de ziektelast bij kinderen weliswaar heeft verminderd, maar dat het aantal gevallen van kinkhoest bij volwassenen almaal toeneemt. Deze toename vergroot de kans op transmissie van de infectie naar zuigelingen wat mogelijk negatieve gevolgen heeft voor de effectiviteit van vaccinatie bij deze kinderen. Dit benadrukt het belang van aanvullende maatregelen om ernstige kinkhoest bij jonge kinderen te voorkómen. Deze maatregelen kunnen ofwel gericht zijn op het onderbreken van de overdracht van kinkhoest naar baby’s of op het direct verbeteren van de immuniteit van pasgeboren tegen kinkhoest, bijvoorbeeld door direct na de geboorte te vaccineren.

Infectiefrequentie
Surveillance artefacten, zoals toegenomen aandacht voor kinkhoest en verbeterde diagnostiek hebben mogelijk bijgedragen aan de toename van het aantal kinkhoestmeldingen. Omdat bijna iedereen antistoffen tegen pertussistoxine ontwikkelt na infectie met B. pertussis, kan het aantal kinkhoestinfecties het meest betrouwbaar worden geschat door de prevalentie van hoge concentraties IgG tegen pertussis toxine (IgG-Ptx) in sera te bepalen. Deze seroprevalentie wordt immers niet beïnvloed door bovengenoemde surveillance artefacten.

Aangezien moeders met kinkhoest op het moment van de bevalling een grote kans hebben om hun pasgeboren baby te infecteren, hebben we de prevalentie van kinkhoestinfecties bij zwangeren bepaald in hoofdstuk 5. In totaal vertoonden 20 (6,3%) van de 315 zwangere vrouwen in onze studie serologisch bevestigde B. pertussis infectie tijdens of kort vóór de zwangerschap. Dit percentage is veel hoger dan het aantal gemelde gevallen in deze leeftijdsgrup (hoofdstuk 2).

De infectiefrequentie van kinkhoest in de algemene bevolking in 2006-07 hebben we vastgesteld door IgG-Ptx concentraties te bepalen in 7.903 sera afkomstig uit een representatieve dwarsdoorsnede van de Nederlandse bevolking (hoofdstuk 6). De hieruit verkregen leeftijdsspecifieke seroprevalentie (0-79 jaar) is vergeleken met de seroprevalentie verkregen uit een vergelijkbare nationale studie uitgevoerd in 1995-96 én met de incidentie berekend op basis van wettelijke meldingen in beide periodes. In 2006-07 bleek naar schatting 9,3% van de personen ouder dan 9 jaar een kinkhoestinfectie te hebben doorgemaakt in het voorgaande jaar, ten opzichte van 4,0% in 1995-96. Opvallend is dat de trends in de seroprevalentie vergelijkbaar zijn met trends in de gerapporteerde incidentie. Dat duidt erop dat fluctuaties in de gerapporteerde incidentie een goede weergave zijn van trends in de circulatie van kinkhoest.
Transmissieroutes
Om inzicht te krijgen in de meest voorkomende transmissieroutes voor kinkhoest, hebben we voor de periode 1996-2006 de leeftijdsdistributie van kinkhoest in Nederland onderzocht (hoofdstuk 7). De bevinding dat de jaarlijkse toename van kinkhoest bij volwassenen gelijktijdig plaatsvindt met de jaarlijkse toename bij jonge kinderen duidt erop dat de infectie vaak wordt overgedragen binnen en tussen deze leeftijdsgroepen. Deze mogelijkheid werd bevestigd door de resultaten van een huishoudonderzoek naar transmissie van kinkhoest binnen gezinnen (BINKI-studie: Baby’s geINfecteerd met KInkhoest). In deze studie (hoofdstuk 8) hebben we onderzocht wie de meest waarschijnlijke bronnen van kinkhoestinfectie bij jonge baby’s zijn. Deze zuigelingen zijn immers het meest kwetsbaar voor een ernstige kinkhoestinfectie. Met behulp van PCR, kweek en serologie zijn 560 gezinscontacten van 164 baby’s die vanwege kinkhoest in het ziekenhuis waren opgenomen, getest op kinkhoest. Van de gezinscontacten had 53% een laboratoriumbevestigde kinkhoestinfectie. De meest waarschijnlijke bron van infectie bij de baby was een broertje/zusje (in 41% van de gevallen), de moeder (38%), of de vader (17%). Deze gegevens laten zien dat selectieve vaccinatie van personen die veelvuldig in contact komen met jonge kinderen (de zogenaamde ‘cocooning-strategie’) de overdracht van kinkhoest naar zuigelingen zal verminderen. Omdat toekomstige ouders regelmatig contact hebben met gezondheidszorg-instanties en omdat ze gemotiveerd zullen zijn om hun baby optimaal te beschermen, verdient het de aanbeveling de ‘cocooning strategie’ op korte termijn in te voeren.

Toekomstige vaccinatie-strategieën
De huidige kinkhoestvaccins beschermen een individu slechts tijdelijk tegen kinkhoest en na een paar jaar neemt het risico op (transmissie van) infectie weer toe. Daarom evalueerden we (hoofdstuk 9) de mogelijkheid om de immuuniteit van de jonge zuigelingen te verbeteren door de moeder tijdens de zwangerschap te vaccineren. Antistoffen van de moeder worden via de placenta naar het kind getransporteerd, waardoor vaccinatie van de moeder tijdens de zwangerschap de baby kan beschermen vanaf het moment van de geboorte. Echter, omwille van ethische, technische en juridische dilemma’s is het moeilijk om de werkzaamheid en veiligheid van deze strategie in klinische studies te onderzoeken.

In de algemene discussie van dit proefschrift (hoofdstuk 10) doen we aan de hand van de meest waarschijnlijke oorzaken van de grote toename van kinkhoest voorstellen voor maatregelen om de bestrijding van kinkhoest te verbeteren.

In de literatuur worden verschillende verklaringen voor de toename van kinkhoest gegeven: toegenomen aandacht, verbeterde diagnostiek, suboptimale vaccins, wegebende immuniteit en pathogeen-adaptatie. Door het combineren van klinische-, pathogeen- en immuunsurveillance
gegevens concluderen wij dat de toename van kinkhoest in Nederland niet enkel kan worden toegeschreven aan toegenomen aandacht en/of verbeterde diagnostiek. De verdubbeling van de seroprevalentie toont aan dat de circulatie van de ziekteverwekker in de afgelopen decennia ook is toegenomen. Het gelijktijdig opkomen van nieuwe B. pertussis stammen en de stijging van de seroprevalentie onder volwassenen suggereert dat de ziekteverwekker zich heeft aangepast aan gastheren met wegebbende immuniteit, waardoor een groot bacterieel reservoir is ontstaan bij volwassenen.

Dankwoord
Dankwoord

De berg is bedwongen, het boekje is af! Maar zo’n berg werk verzet je niet alleen. Ik wil dan ook een aantal mensen bedanken die mij geholpen hebben deze top te bereiken.

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Een paar collega’s wil ik in het bijzonder bedanken:
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Curriculum Vitae
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Sabine Christine de Greeff was born the 2nd of May 1978 in Nijmegen, the Netherlands. After passing her secondary school exams at the Eckartcollege in Eindhoven in 1996, she started the study Nutrition and Health at Wageningen University. In her first research internship she studied the possible virological cause of acute respiratory infections in elderly. For her second research internship she went to Nanjing, China, to study the relation between excessive iodine intake and cognitive and physical development in children. In November 2000, she graduated with distinction with a major in epidemiology. Since 2001, she works as an epidemiologist at the Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM). In collaboration with researchers within and outside the RIVM she evaluates the effectiveness of the National Immunization Programme (NIP) and performs specific studies to investigate the effects of the NIP on pathogens, diagnostics and clinical outcomes in the population. Her research is mainly focussed on pertussis, meningococcal and pneumococcal disease. In 2002, she was a member of the project team that coordinated the nationwide vaccination campaign against meningococcal C disease. In 2007, she was awarded “young investigator of the year” by the CIb. Her work resulted in several (inter)national publications and has contributed to policy making regarding the NIP in the Netherlands.