Pertussis: a concise historical review including diagnosis, incidence, clinical manifestations and the role of treatment and vaccination in management

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Pertussis (whooping cough) is a highly contagious acute bacterial disease involving the respiratory tract and is caused mainly by *Bordetella pertussis*. Since the last decade many developed countries experience a re-emergence of pertussis, even countries that have had high vaccination coverage for many years. In this study we review the historical facts, clinical manifestations, microbiology, pathogenesis, host defences, epidemiology, transmission, immunity, diagnosis, treatment and prevention.
Finally we describe some new insights in diagnosis, incidence and clinical manifestations. Special attention is given to one-point serology, re-infection with *Bordetella pertussis*, the decay of immunoglobulin G against pertussis toxin after *Bordetella pertussis* infection in different age groups, the infection frequency in the general population and the occurrence of mixed infections.

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Introduction

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

History

The history of whooping cough starts, according to the literature, with the description by Guillaume de Baillou (1538–1616) [1] of an epidemic in 1578 in France, published for the first time only in 1640 by his nephew. Was the disease not known before, or known maybe by other names and in different countries by different names? Kohn [2] suggests that the description of the *Perinthus cough* by Hippocrates (around 400 B.C.) might possibly be whooping cough or a mix with other diseases such as viral respiratory infections. In the Oxford English Dictionary [3] *kinkehost* is mentioned in Reginald's Vita Godrici from around 1190. In the *Middelnederlandsch woordenboek* [4] (dictionary of medieval Dutch) it is suggested that *gisschen* might be an early eastern Dutch word for whooping cough in the first half of the fourteenth century. In his History of Pediatrics in the Netherlands [5] van Lieburg refers to the Miracle book of the St Jan's Cathedral in 's Hertogenbosch, in the southern part of the Netherlands, in which a pilgrimage is described to the statue of the Holy Mary because of the recovery of a boy from *kychoest*, in 1383. Nils Rosén von Rosenstein [6] from Sweden has stated, not knowing when the disease came to his country, that in France it first appeared in 1414, without giving a source. In Schiller-Lübben's *Mittelniederdeutsches Wörterbuch* [7] (dictionary of medieval

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German) *kinkhoste* is found in a source from 1464. Dodonaeus (1517–1585) in his *Cruijde boeck* [8] (book of herbs) from 1554 already describes cures for the *kieckhoest*!

De Baillou called it *quinta*, referring to Hippocrates [1]. *Coqueluche*, the present name in French for whooping cough, was then the common name for influenza [9]. Holmes [10] reports that pertussis was called *chyne-cough* in England as early as 1519. Cherry and Heininger [11] say it was called the *kink* (in Scottish synonymous with fit or paroxysm) and *kindhoest* (a Teutonic word meaning child's cough) in the Middle Ages. Nils Rosén von Rosenstein [6] calls it in his book about paediatric diseases from 1798 *Keichhussten*. In a *JAMA* editorial [12] names such as *tosse canina* (dog's bark, Italy), *Wolfshusten* (howling of wolves) and *Eselshusten* (braying of donkeys) (both from Germany), and *chincough* (boisterous laughter, Old English) are given. In Chinese it is called "cough of 100 days" [13]. In Dutch it is called *kinkhoest*, coming from old names as *kinkhôste*, *kichhoest*, *keichhusten*, as van Esso describes [14]. In the Dictionary of the Dutch Language (*Woordenboek der Nederlandsche Taal*) [15] the same names are given, and others as *kie(c)khoest*, *kijkhoest*, *kikhoest*. Also it refers to Dodonaeus (1517–1585) who called the disease *kich*, or *kinchoest* in a latter edition of his "*Cruydt-Boeck*" from 1608 [16].

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

Clinical manifestations

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

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

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

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

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

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

Microbiology

The genus *Bordetella* contains species of related bacteria with similar morphology, size, and staining reactions. To date there are eight species known of *Bordetella*: *B. pertussis* [27], *B. parapertussis* [28,29], *B. bronchiseptica* [30], *B. avium* [31] (formerly designated *Alcaligenes faecalis*), *B. hinzii* [32,33] (formerly designated *A. faecalis* type II), *B. holmesii* [34], *B. trematum* [35] and *B. petrii* [36]. *Bordetella pertussis*, *B. parapertussis* and *B. bronchiseptica* are genomically closely related. The first four are respiratory pathogens. *Bordetella pertussis* is an obligate human pathogen. *Bordetella pertussis* was long considered the sole agent of whooping cough. A mild, pertussis-like disease in humans may be caused by *B. parapertussis* and occasionally by *B. bronchiseptica*. *Bordetella parapertussis* appears both in humans and animals. The natural habitat of *B. bronchiseptica* is the respiratory tract of smaller animals such as rabbits, cats, and dogs. Human infections with

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

Adenylate cyclase toxin (ACT)
An extracytoplasmic enzyme that impairs host immune cell function by elevating the levels of intracellular cAMP; by virtue of its hemolysin function it may contribute to local tissue damage in the respiratory tract
Bordetella resistance to killing factor (Brk)
A 32-kDa outer-membrane protein. An adhesin that also provides resistance to killing by the host's complement system
Filamentous hemagglutinin (FHA)
A cell surface protein. Promotes attachment to respiratory epithelium. Agglutinates erythrocytes *in vivo*. Antibodies to FHA protect against respiratory tract challenge but not against intracerebral challenge in mice
Fimbriae
Two serologic types (types 2 and 3). Antibody to specific types causes agglutination of the organism. Organisms may contain fimbriae 2, fimbriae 3, fimbriae 2 and 3, or neither fimbriae 2 nor fimbriae 3. Fimbriae may play a critical role as adhesins
Heat-labile toxin (also called dermonecrotic toxin)
Cytoplasmic protein that causes ischemic necrosis at dermal injection site in laboratory animals. It may contribute to local tissue damage in the respiratory tract
Lipopolysaccharide (LPS) (endotoxin)
An envelope toxin with activities similar to endotoxins of other Gram-negative bacteria. A significant cause of reactions to whole-cell pertussis vaccines. Antibody to lipopolysaccharide causes agglutination of the organism. Associated with fever and local reactions in mice
Pertactin (PRN)
A 69-kDa outer-membrane protein that is an important adhesin. Adenylate cyclase-associated. Antibody to pertactin causes agglutination of the organism and protects against respiratory tract challenge in mice
Pertussis toxin (PT) (also called lymphocytosis-promoting factor)
A classic bacterial toxin with an enzymatically active A subunit and a B oligomer-binding protein. pertussis toxin promotes attachment to respiratory epithelium, sensitization to histamine, elicits lymphocytosis, enhances insulin secretion, and stimulates adjuvant and mitogenic activity. It is an extracellular envelope protein. It causes T lymphocyte mitogenesis, stimulates interleukin-4 and IgE production, inhibits phagocytic function of leukocytes, and it causes cytopathic effect on Chinese hamster ovary cells. Antibodies to pertussis toxin protect against respiratory tract and intracerebral challenge in mice
Tracheal colonization factor (TCF)
A proline-rich protein that functions predominantly as an adhesin in the trachea
Tracheal cytotoxin (TCT)
A disaccharide-tetrapeptide derived from peptidoglycan. Causes local tissue damage in the respiratory tract, and ciliary stasis
Type III secretion system (bscN)
Several not yet specified proteins that secrete effector proteins into host cells

B. bronchiseptica are rare and occur only after close contact with carrier animals, no human-to-human transmission occurs. Most patients with severe disease caused by *B. bronchiseptica* are immunocompromised [37]. *Bordetella avium* and *B. hinzii* are important in birds. *Bordetella hinzii*, and *B. holmesii* are found in blood cultures from immunocompromised patients. *Bordetella trematum* and *B. petrii* have been recently discovered, *B. trematum* in wounds in humans, *B. petrii* (an anaerobic species) in a bioreactor.

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

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

Bordetella pertussis has affinity for the mucosal layers of the human respiratory tract; it has different antigenic or biologically active components (Table 1) but their exact chemical structure and location in the bacterial cell are known only in part.

Pathogenesis

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

Host defences

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

Pertussis toxin is assumed to be one essential protective immunogen, but numerous findings indicate that other

components, such as filamentous hemagglutinin, heat-labile toxin, agglutinogens, outer membrane proteins, and adenylate cyclase toxin, may also contribute to immunity after infection or vaccination [44–46]. In addition, it was recently shown that antibodies to pertactin, but not to pertussis toxin, fimbriae, or filamentous hemagglutinin, are crucial for phagocytosis of *B. pertussis* [47]. The immunogenicity of these substances may be significantly increased by the presence of pertussis toxin [48]. This synergism indicates that pertussis toxin could function as an adjuvant to a variety of protective antigens of *B. pertussis*. The defence mechanisms are both non-specific (local inflammation, increase in macrophage activity, and production of interferon) and specific (proliferation of specific B and T cells) [49].

The nature of immunity in whooping cough is, however, incompletely understood. A role for circulating antibody in immunity is indicated by the correlation between protection of human vaccinees and their antibody titres [44–46]. However, effective immunity does not necessarily depend on the presence of protective antibodies, and immunity to whooping cough may therefore be mediated essentially by cellular mechanisms [49,50]. This cell-mediated immunity may be considered the crucial carrier of long-term immunity, and titres of specific humoral antibodies may diminish over time.

Epidemiology

Worldwide, *B. pertussis* causes some 20–40 million cases of pertussis per year, 90% of which occur in developing countries, and an estimated 200 000–400 000 fatalities each year [11,13,51]. Since the last decade many developed countries have experienced a re-emergence of pertussis, even countries that have had high vaccination coverage for many years. Because of waning natural and vaccine-induced immunity older children and adults are susceptible to infection again. Therefore it is assumed that infection frequency is probably highest in adolescents and adults and consequently those age groups are the main source of infection for infants [52].

In many countries there has been an increase in the incidence of *B. pertussis* infection since the 1990s [53–57]. In the Netherlands in 1989–1994 the mean incidence on the basis of notification and serology was 2.4 and 2.3 per 100 000 per year. In 1996 for example, there was in the Netherlands a steep increase in notifications (27.3/100 000), positive serology, hospital admissions and even deaths [57–59]. Since then the incidence has remained higher than before 1996 (Fig. 1).

Although in older literature there are reports of re-infection with whooping cough, there are no reports on proven re-infection [41,60–64].

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

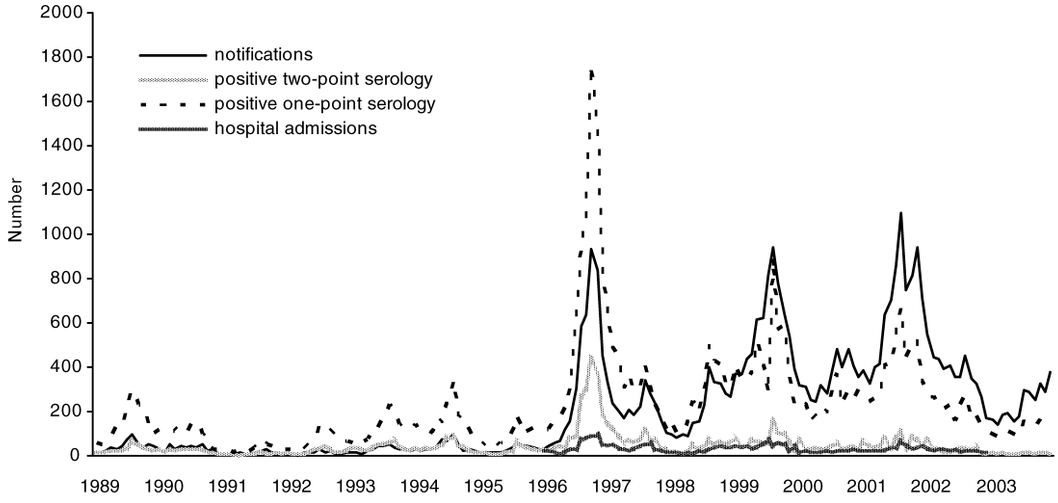

Fig. 1. Pertussis in the Netherlands from 1989 to 2004: notifications, positive two-point serology, positive one-point serology; hospital admissions from 1996 to 2004, based on first day of illness [52]. Note: Before 1996 all serological tests for pertussis were performed at the LIS-RIVM. However, since 1998 at least three of the 16 regional Public Health Laboratories and also some other (hospital) laboratories have started to perform serology with commercial available assays. Consequently, the population coverage of serological surveillance based on serological data of LIS-RIVM is now estimated to have decreased from 100% in 1996 to less than 50% in 2002.

the vaccine strains, showing polymorphism in pertussis toxin and pertactin [65]. This might have contributed to the re-emergence of pertussis in the Netherlands.

Transmission

In most countries *B. pertussis* is endemic with superimposed epidemic cycles. These cycles occur approximately every 4 years in vaccinated populations and approximately every 2–3 years in non-vaccinated populations, although in the Netherlands the incidence is higher now (every 2–3 years) compared to the period prior to the epidemic in 1996–1997 (every 4 years) [52,66]. Most infections occur from July to October. Pertussis is very contagious. It is transmitted obligatorily from human to human by direct contact with discharges from respiratory mucous membranes of infected persons primarily via droplets by the airborne route. The mucous membranes of the human respiratory tract are the natural habitat for *B. pertussis* and *B. parapertussis*. Most infections occur after direct contact with diseased persons specifically, by inhalation of bacteria-bearing droplets expelled in cough spray. The patient is most infectious during the early catarrhal phase, when clinical symptoms are relatively mild and non-characteristic. Subclinical cases may have similar epidemiologic significance. Healthy transient carriers of *B. pertussis* or *B. parapertussis* are assumed to play no significant epidemiologic role. Chronic carriage by humans is not documented.

Immunity

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

Although vaccination has caused a firm decrease in incidence and mortality over the years, occasional local epidemics do occur. The disease is especially dangerous in the first 6 months of life. There seems no influence of the season or climate on the morbidity rate. Older persons (i.e., adolescents and adults), and those partially protected by the vaccine may become infected with *B. pertussis*, but usually have milder or asymptomatic disease [67]. Pertussis in these persons may present as a persistent ($>$ 7 days) cough, and may be indistinguishable from other upper respiratory infections. Inspiratory whoop is uncommon. In some studies, evidence for a *B. pertussis* infection was found in 25% or more of adults with cough illness lasting $>$ 7 days [68,69]. Even though the disease may be milder in older persons, these infected persons may transmit the disease to other susceptible persons, including unimmunized or underimmunized infants. Adults are often found to be the first case in a household with multiple pertussis cases [70,71].

Diagnosis

Whooping cough is a clinical diagnosis according to WHO criteria [53], as established in 2000: a case diagnosed by a physician, or a person with a cough lasting at least 2 weeks with at least one of the following symptoms: paroxysms (i.e., fits) of coughing, inspiratory 'whooping' or post-tussive vomiting (i.e., vomiting immediately after coughing) without other apparent cause. Criteria for laboratory confirmation are: isolation of *B. pertussis* or detection of genomic sequences by polymerase chain reaction (PCR) or positive paired serology (i.e., at least a fourfold increase) [72].

Only since Bordet and Gengou [27] in 1906 cultured *B. pertussis* we could be sure about the diagnosis. Recovery of *B. pertussis* is the Gold Standard. But culturing *B. pertussis* is not very easy. *Bordetella* can be cultured from nasopharyngeal swabs or nasopharyngeal secretions. The sensitivity of the culture depends mainly on the technique of taking the nasopharyngeal swabs (calcium alginate or Dacron) or secretions, direct inoculation of nasopharyngeal swab material onto special freshly prepared media (Bordet-Gengou or Regan-Lowe) for primary isolation and immediate aerobic incubation in a stove. *Bordetella pertussis* grows slowly, thus it is recommended to extend incubation time from 7 to 14 days.

The newest test for detection of *B. pertussis* and *B. parapertussis* is by PCR, a very specific test and more sensitive than culture. Nasopharyngeal swabs are shaken in fluid, incubated and amplified. The final PCR product is analysed by gel electrophoresis and hybridization. In later stages of the disease PCR testing is more often positive than culture, and in patients treated with antibiotics or in vaccinated patients [73]. The PCR yield is about 2.4-fold higher than culture. The poor performance of culture may be due the fastidious nature of *B. pertussis*, but it is also possible that by PCR *B. pertussis* DNA is detected in samples in which the

organisms have become non-viable. In patients with clinical symptoms of *B. pertussis* infection and positive serology sensitivity of PCR and culture is low (21 and 7%, respectively) but specificity of both is 98% [73].

In adolescents and adults culture or PCR is not useful when disease duration is longer than 3–4 weeks [73]. In contrast in non-vaccinated or partially vaccinated young children culture or PCR is useful in any stage of the disease as they have an immature mucosal immune response and therefore a slower eradication of the bacteria.

As the usefulness of PCR and culture declines with increasing disease duration, serology becomes more important. Already in 1911 Bordet and Gengou published the first serological methods, detecting agglutinating antibodies to whole *B. pertussis* cells [74]. This remained the hallmark of pertussis serology for more than 70 years [75]. In the 1980s various enzyme immunoassays were developed, and presently immunoglobulin G against pertussis toxin (IgG-PT) is the most used and validated test to prove *B. pertussis* infection [72,75]. IgG-PT is only produced after infection with *B. pertussis*, and not other *Bordetella* species, nor are any cross-reactions described. In newborn infants and after vaccination against *B. pertussis* IgG-PT should be looked at with caution because of transplacental transfer or induction by vaccination [72]. After the fourth vaccination with the Dutch vaccine there is only a temporary and small increase and in both instances there is a fast decrease [76]. However, other whole-cell vaccines and acellular vaccines might induce higher IgG-PT levels [45,77]. Antibodies against other antigens such as pertactin, fimbriae and filamentous hemagglutinin (FHA) are also available, but less validated, and are also produced in reaction to infections with other *Bordetella* species and perhaps other related bacteria. IgG-PT, appearing as late as week 3 of illness reaches its peak approximately 4.5 weeks after infection, but is retarded in very young children (< 1 year). Serology for *B. pertussis* is considered positive by the finding of a significant, at least a fourfold increase of IgG antibodies to pertussis toxin (IgG-PT) in paired sera to a level of at least 20 U/ml. This hampers the diagnosis of *B. pertussis* infection, since many patients present themselves later in their disease, having already high levels of IgG-PT without showing a significant increase.

Differential diagnosis

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

Treatment and prevention

Although immunization against *B. pertussis* infection has caused a great reduction in the incidence of pertussis, outbreaks still occur, even in countries with high vaccination coverage. Erythromycin, 40–50 mg/kg per day for 10–14 days, usually considered the treatment of choice, will eliminate viable *B. pertussis* organisms from the respiratory tract within a few days [11,78–81]. A 7-day course of erythromycin has proven to be as efficacious as a 14-day course [82]. Newer macrolides such as azithromycin, 10 mg/kg per day for 3 or 5 days [79,83], or 10 mg/kg the first day and 5 mg/kg per day for 4 days [81,83], or clarithromycin, 10–15 mg/kg per day for 7 days [79,80] have also been shown to be effective in the treatment of pertussis with fewer side effects than erythromycin. Erythromycin-resistant strains of *B. pertussis* have been isolated, but this seems to be very uncommon [84,85]. Although rare, the use of erythromycin in young infants is associated with hypertrophic pyloric stenosis [86,87]. An alternative to erythromycin is trimethoprim–sulfamethoxazole, 6–10 mg trimethoprim/kg per day for 14 days [88]. Fluoroquinolones have good *in vitro* activity against both *B. pertussis* and *B. parapertussis* and may be useful in the treatment of *B. pertussis* infection, although there are no supporting clinical data at present [89].

Human hyperimmune pertussis globulin is still used occasionally [90,91]. Further treatment is symptomatic. High altitude, flying or hypobaric therapy have been suggested as effective treatments for the cough [92–94].

During the paroxysmal phase of the disease, eradication of the bacteria by antimicrobial drugs, such as erythromycin, will not significantly change the clinical course, although there is some clinical evidence that some macrolides might reduce cough [95].

Although it is better for susceptible children (unimmunized children without a history of whooping cough) to avoid contact with pertussis patients during the first 4 weeks of their illness, this is often difficult to achieve. Exposed unimmunized children are given a macrolide for 10 days after contact is discontinued or after the patient ceases to be contagious. Exposed immunized children younger than 4 years are most probably protected but protection may be enhanced by macrolides or by a booster dose of acellular pertussis vaccine [96].

Vaccination

Currently, approximately 80% of the world's children are vaccinated against pertussis, most of whom have received

the diphtheria–tetanus–whole cell pertussis combination [51].

Pertussis vaccine is produced from smooth forms (phase I) of the bacteria as a killed whole-cell vaccine. General vaccination was introduced in the Netherlands in 1952. Furthermore, since 1 January 1999 the primary vaccination for pertussis has been advanced. From that time children are vaccinated at the age of 2, 3, 4 and 11 months, instead of 3, 4, 5 and 11 months. In November 2001 a booster vaccination with an acellular vaccine, comprised of pertussis toxin, pertactin and filamentous hemagglutinin, was introduced in the National Immunization Programme at the age of 4 years [52].

Owing to a relatively mild course of disease and to occasional complications after vaccination, it has been argued that general vaccination with the whole-cell vaccine is no longer justified. Therefore acellular pertussis vaccines have been developed. These vaccines are composed very differently and contain various amounts of structural components from the bacteria. Components available for vaccine production include pertussis toxin (which is detoxified), filamentous hemagglutinin, pertactin, and fimbrial antigens 2 and 3. Since 2000 4-year-old children in the Netherlands are given a booster with acellular vaccine. Recent data suggest that after primary vaccinations of infants these vaccines can convey similar levels of protection as the whole-cell vaccine. Thus, acellular vaccines have also been licensed for primary vaccination [97].

New insights in diagnosis, incidence and clinical manifestations

In 1996 there was an outbreak of pertussis in the Netherlands, both in vaccinated and in non-vaccinated people of all ages. Many questions arose as to what the cause of this sudden increase in *B. pertussis* infection was. Among others the question arose whether vaccination or previous natural infection with *B. pertussis* guaranteed lifelong protection. We demonstrated recently that children, vaccinated or not, may have serologic evidence of re-infection with *B. pertussis* [98]. Although it is known that people may suffer from re-infection with *B. pertussis*, these patients were, to our knowledge, the first in whom symptomatic re-infection with *B. pertussis* has definitely been proven, 3.5–12 years after the first infection, by laboratory confirmation of both episodes. But it is obvious from these patients that their clinical symptoms did not necessarily match with typical pertussis infection. In this study it was shown that the severity and duration of respiratory symptoms in patients with proven and relatively early re-infections with *B. pertussis* increased with time elapsed since the first infection. In our opinion *B. pertussis* infection should be considered in patients with symptoms of typical or atypical whooping cough, irrespective of age, their vaccination status or previous whooping cough.

As stated before, criteria for laboratory confirmation of *B. pertussis* infection are: isolation of *B. pertussis* or detection of genomic sequences by PCR or significant, at least a fourfold increase of IgG-PT in paired sera to a level of at least 20 U/ml. Because many patients visit their physician after weeks of coughing, culture or PCR are less sensitive and serology may already show high levels of IgG-PT without further significant increase. Thus the question arises whether one-point serology could be a useful tool in the diagnosis of *B. pertussis* infection. Since there are no cut-off values in one-point serology as proof of actual or recent *B. pertussis* infection it would be opportune to develop such cut-off values. Therefore a study was performed in four different patient groups [99].

(1) IgG-PT data of a cross-section of the general Dutch population ($n = 7756$).
(2) Patients with serologically confirmed pertussis ($n = 3491$): clinical suspicion of pertussis confirmed by the detection, in paired sera, of at least a fourfold increase of IgG-PT to ≥ 20 U/ml.
(3) Patients with typically symptomatic infection with *B. pertussis* and their longitudinal sera ($n = 57$).
(4) Patients with PCR and/or culture-proven pertussis ($n = 89$).

The results showed that an IgG-PT level of at least 100 U/ml (Dutch Units) is a specific tool in laboratory confirmation by one-point serology of patients with a suspected pertussis infection in the Netherlands, and might be in other countries too, and that, independently of age, these levels are diagnostic of very recent or actual infection with *B. pertussis*. Such levels are present in less than 1% of the population and are reached in most pertussis patients within 4 weeks of disease onset. High IgG-PT levels persist only temporarily. The levels decreased within less than 1 year to a level below 100 U/ml after natural infection with *B. pertussis* for almost all patients who had had high IgG-PT levels. The regression model used in this study predicts that peak levels > 100 U/ml occur 4–8 weeks after infection, that in the declining phase a level of 100 U/ml is reached in 4.5 months after onset infection and a level of < 40 U/ml is reached within 1 year of onset of the disease. It was also shown that the number of patients with IgG-PT levels ≥ 100 U/ml is considerably larger (4.5-fold) than the number of patients with at least a fourfold increase in IgG-PT. The control group in this study consisted of a large number of participants from a population-based study so this probably guarantees a better representative sample than in studies with smaller groups. Thus, we believe that high IgG-PT levels > 100 U/ml could provide a useful

laboratory tool for the diagnosis of pertussis in both the individual patient and in epidemiological studies. Baughman *et al*. [100] found a cut off $\geq$ 94 U/ml (FDA units) diagnostic for a recent infection with *B. pertussis*. Since 100 Dutch units equals 125 FDA units [101] this cut off is considerably lower than the one found by the Melker *et al*. [99].

The next question is what is the natural course of IgG-PT after infection. Accordingly, it is necessary to gain insight into the rise, peak and decline of IgG-PT after natural infection with *B. pertussis.*

Our group used different ways of evaluating the decline of IgG-PT after natural infection with *B. pertussis* [99,102,103]. These three methods reflect gradual improvement and advances in understanding. In response to an infection, IgG titres typically show a rapid increase, followed by a steady, slow decline over several years. IgG-PT responses appear to show considerable variation among individual patients.

The first model analyses the association between ^{2}log IgG-PT levels and time in ^{2}log days, fitting a straight line only accounting for the decaying phase of the response. This requires omission of data from the initial rapid increase of IgG-PT [99]. In this study the rapid increase of IgG-PT in the first weeks after illness was not taken in account, nor were effects of age or vaccination status. Since there is no clearly defined criterion by which observations can be excluded, the following study [102] continued with a response model, which includes the initial rising phase. The model that was used, a skewed hyperbola, has asymptotically linear decline on a log–log scale at long times from infection. In this model there seemed a significant effect of age or vaccination status, but because numbers of adults and unvaccinated infants were small and because there was a wide variation of data it remained unclear whether this was clinically relevant.

For any individual patient the amount of information in this study was limited: only a few measurements at best. Although the previous regression model adequately fitted the data, this model did not help much in interpreting the observed immune responses. For that reason a biologically based model was developed [103]. The longitudinal responses are described with a dynamic model of the interaction between bacteria and the immune system. This so-called predator–prey model is the simplest possible model for the interaction between host and pathogen.

In this study, combining data from patients aged 0–94 years, there were no significant differences found in rise, peak and decline of IgG-PT between different age groups. There seems a tendency to age-related differences where older people tend to have a more rapid increase, a higher peak and a faster decline after infection than in younger age groups, which could be caused by immunological memory.

Since *B. pertussis* infection is especially dangerous in young children, not or partially vaccinated, and since the main source of infection for these children are adults with often atypical clinical manifestations [104] it is important to gain insight into the incidence of *B. pertussis* infection [103]. Is it possible, once knowing the natural course of IgG-PT, to calculate the incidence of *B. pertussis* infection in the Netherlands in different age groups from available surveillance data on IgG-PT levels in the general population?

Earlier de Melker *et al*. showed that an IgG-PT level of at least 100 U/ml is present in less than 1% of the population [99]. De Melker *et al*. [105], using the statistical model described by Teunis *et al*. [102] on the IgG-PT data of a cross-section of the general Dutch population ($n = 7756$) [99] showed that *B. pertussis* infections occur frequently in the Dutch population, particularly in adults for whom the reported incidence is very low. On average the estimated incidence of infection was 6.6% per year for 3–79-year-olds. The annual incidence of notified cases was 0.01%. The age distribution of all infections differs notably from the age distribution of notified cases. Therefore we suggest that vaccination strategies should not be based on notification data but on knowledge about the circulation of *B. pertussis* in different age groups and contact patterns between age groups. Especially for the young, not or incompletely vaccinated children it is important to develop vaccination strategies focused on adolescents and adults as they are the most important source of infection of these infants [106].

From other studies [17–22,24] it is known that, in some patients there may be evidence of other pathogens involved in the pertussis syndrome besides *B. pertussis* and in others of pertussis-like complaints without proof of *B. pertussis* infection. In a retrospective study for mixed infections we found that in 28% (23/82 patients) there was evidence of possible mixed infection with other pathogens such as parainfluenza virus, respiratory syncytial virus, *Mycoplasma pneumoniae*, adenovirus or influenza virus [107]. Then, to investigate the role of different respiratory pathogens in prolonged coughing in children, to confirm the data of the previous study and to analyse the clinical impact of mixed infections of *B. pertussis* with other respiratory pathogens we performed a prospective study in patients aged 0–18 years with coughing symptoms lasting 1–6 weeks [108]. In 91 of the 136 included patients one ($n = 49$, 36%) or more ($n = 42$, 31%) possible respiratory pathogens were found. The most frequent pathogens encountered were rhinovirus (43 patients, 32%), *B. pertussis* (23 patients, 17%) and respiratory syncytial virus (15 patients, 11%). In the 42 patients with a mixed infection the most frequent combination was *B. pertussis* and rhinovirus ($n = 14$).

Infections with more than one pathogen occurred during the whole year regardless of the season. However, we could not demonstrate signs of enhanced disease severity in children with more than one pathogen, although children with more than one pathogen were significantly older than those with none or one pathogen. There were no clinical data found that discriminated between pathogens, whether pathogens were found or not, or differences in treatment.

Conclusion

Bordetella pertussis infection is still a burden especially in young children. Understanding the pathogenesis and immunity better will provide opportunities to develop new strategies in preventing disease in young children and to control pertussis epidemics.

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