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Seroepidemiology of diphtheria, tetanus, poliomyelitis and pertussis

Evaluation of the National Immunisation Programme in the Netherlands

Hester de Melker

STELLINGEN

behorend bij het proefschrift
'Seroepidemiology of diphtheria, tetanus, poliomyelitis and pertussis.
Evaluation of the national immunisation programme in the Netherlands'
van Hester de Melker

1. Het is niet (meer) gerechtvaardigd de ziekten uit het Rijksvaccinatieprogramma als *kinderziekten* aan te duiden. *(Dit proefschrift)*

2. In seroepidemiologisch onderzoek naar de effecten van vaccinatie heeft (gedocumenteerde) vaccinatiestatus grote waarde. *(Dit proefschrift)*

3. Onderzoekers moeten zich er van bewust zijn dat het overhalen van mensen om mee te werken aan onderzoek niet hoeft te leiden tot betere representativiteit. *(Dit proefschrift)*

4. Het is onjuist om de cumulatieve incidentie van *Bordetella pertussis* infecties direct te schatten uit de leeftijdspecifieke prevalentie van IgG antistoffen tegen pertussis toxine. *(Dit proefschrift)*

5. Vaccinatie kan het slachtoffer worden van haar eigen succes.

6. 'Meten is weten' is geen universele waarheid.

7. Het op de markt brengen van schoonmaakmiddelen die 99,9% van de bacteriën doden, verhoogt de kans op smetvrees voor de 0,1% niet-gedode bacteriën.

8. Alhoewel immuniteit tegen intolerant gedrag goed van pas kan komen, is het verbeteren van de algemene tolerantie een veel beter uitgangspunt.

9. De kracht van een (hockey)team moet niet alleen worden vastgesteld aan de hand van de positie op de ranglijst.

10. Terwijl sommige gaven van 'zwakbegaafden' verwonderlijk mooi zijn, is het gemis van sommige gaven van '(hoog)begaafden' verwonderlijk vervelend.

11. Het kúnnen maken van keuzes in het leven, zou veel meer als een voorrecht beschouwd moeten worden.

12. Geografische afstand kan ook positief zijn geassocieerd met kwaliteit van communicatie.

13. Distance makes the heart grow fonder!

Wageningen, 10 december 1999

Seroepidemiology of diphtheria, tetanus, poliomyelitis and pertussis

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[D = difterie (diphtheria); K = kinkhoest (pertussis); T = tetanus; P = poliomyelitis]

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ABSTRACT

Seroepidemiology of diphtheria, tetanus, poliomyelitis and pertussis. Evaluation of the National Immunisation Programme in the Netherlands

PhD Thesis. Wageningen University and the National Institute of Public Health and the Environment, Bilthoven, the Netherlands

Hester de Melker

In view of the evaluation of the National Immunisation Programme in the Netherlands the main objectives were to obtain insight into the immunity to diphtheria, tetanus and poliomyelitis, into the occurrence of pertussis and to improve serodiagnosis of pertussis.

In a population-based nationwide sampling, 8359 sera (response 55%) were collected, and to gain assess to orthodox reformed individuals refusing vaccination, in a sample from municipalities with low vaccine coverage 1589 sera (response 52.5%). In the nationwide sample, the prevalence of diphtheria and tetanus antibodies (≥ 0.01 IU/ml in toxin inhibition assay) was 58% and 72.5%, resp. In at least 90% antibodies (titre ≥1:8 in neutralisation assay) against poliovirus types 1, 2 and 3 were measured. For those born after mass vaccination was introduced (<45 years) the prevalence of antibodies to diphtheria, tetanus, poliovirus types 1 and 2 was at least 92.5% and for poliovirus type 3 at least 80%. Diphtheria and tetanus antibodies decreased with age for those born before vaccination was introduced (≥45 years). Only 40% of orthodox reformed individuals had diphtheria and/or tetanus antibodies and less than 70% had poliovirus type 1, 2 and/or 3 antibodies. We concluded that the Dutch immunisation programme induced long-term diphtheria, tetanus and poliomyelitis immunity. While adults are very well protected against poliomyelitis, a great number of adults lack diphtheria or tetanus antitoxin antibodies. These adults might benefit from diphtheria (re)vaccination; however, offering a primary tetanus vaccination to cohorts born before the introduction of vaccination would probably be more effective than routine revaccination. Introduction of *C. diphtheriae* or poliovirus in socio-geographically clustered orthodox reformed groups might constitute a danger of spread of these pathogens.

Pertussis surveillance data from notifications, positive serology and hospital admissions (1976-98) showed a sudden increase in the number of pertussis cases in 1996-97. According to notifications and serology data, the increase among, mostly unvaccinated, children less than 1 year was similar to the increase in hospital admissions. For older, mostly vaccinated, individuals the increase in hospital admissions was relatively small. The increase of reported vaccinated patients of all ages was higher than for unvaccinated patients. We postulated that the proportion of pertussis infections resulting in recognizable symptoms has increased among vaccinated individuals due to a mismatch of the vaccine strain and circulating *B. pertussis* strains.

To investigate at which level IgG antibodies against pertussis toxin (IgG-PT) in a single serum sample are indicative for recent pertussis, IgG-PT was analysed in 7756 population-based sera, in sera of 3491 patients with at least a fourfold IgG-PT increase, in paired sera of 89 patients with positive cultures or polymerase chain reactions and in sera of 57 pertussis patients with a median follow-up of 1.4 years. IgG-PT levels of at least 100 U/ml were present in less than 1% of the population, are reached by most pertussis patients within 4 weeks after disease onset and persist only temporarily. We concluded that such levels are diagnostic for recent or actual infection with *B. pertussis*.

Our results not only show that childhood vaccination should be sustained, but that adult vaccination could be considered. We have to anticipate long-term effects of mass vaccination, such as gaps in immunity as a result of decreased circulation of the pathogens and waning immunity. Epidemiological studies directed towards evaluation of vaccination should continue to provide a scientific basis for vaccination strategy.

Contents

Chapter 1

Introduction

Control of vaccine-preventable diseases

The first part of the twentieth century is regarded as a golden age in public health mainly because of the advances in control of infectious diseases (1,2). With the exception of safe water, no other intervention, not even antibiotics, is as powerful and cost effective in disease prevention as immunisation. As a result of immunisation, smallpox has been eradicated and poliomyelitis eradication is well on the way (2-4).
In the absence of immunisation, an estimated number of 5 million deaths from smallpox, 2.7 million from measles, 2.0 million from neonatal tetanus, 1.0 million from pertussis, 600,000 deaths or paralytic cases of polio and 300,000 deaths from diphtheria would occur worldwide each year (2). Death due to smallpox is prevented and the Children's Vaccine Initiative[1] estimates that at least 60% of otherwise expected deaths from other diseases are prevented (2).

However, the bad news is that infectious diseases are still the leading cause of death worldwide and that diseases are (re)emerging (1,5,6). Even among the vaccine-preventable diseases of childhood included in the Global Program for Vaccines and Immunisation (previously the Expanded Programme on Immunisation), many deaths are not prevented. Nearly 40% of potentially preventable deaths due to pertussis, measles and neonatal tetanus still occur (2).
The disease occurrence is not spread evenly over the world. The control of vaccine-preventable diseases is better in developed countries than in developing countries. However, even in an industrialised country such as the Netherlands, which has high vaccination coverage, epidemics of some vaccine-preventable diseases still occur, and the re-emergence of other vaccine-preventable diseases is possible (7-13).

Factors similar to those thought to be involved in the (re)emergence of infectious diseases in general might play a role in the occurrence of vaccine-preventable diseases (5). For example, the adaptation of the pathogen might have contributed to a pertussis outbreak in the Netherlands and a poliomyelitis outbreak in Finland (5,8,12). The breakdown of public health measures was a major factor involved in the re-emergence of diphtheria in the former Soviet Union (5). As a result of international travel, import and spread of the bacterium was feared in other countries (5,7). A long-term paradoxical effect of mass vaccination concerns the gap in immunity in unvaccinated groups; these groups may be worse off than in the prevaccination era. This could result in outbreaks in these groups similar to those observed for congenital rubella syndrome among Amish people in the United States and for poliomyelitis in orthodox reformed groups in the Netherlands (10,14,15).

[1]Co-sponsoring agencies of Children's Vaccine Initiative: United Nations Children's Fund, United Nations Development Programme, World Bank, World Health Organization, Rockefeller Foundation

The Dutch National Immunisation Programme

The Dutch National Immunisation Programme was introduced in 1952, offering diphtheria, tetanus and pertussis vaccination (DTP), and since 1957, poliomyelitis vaccination (IPV) to all children born from 1945 onwards. Nowadays, vaccination against measles, mumps, rubella (MMR) and *Haemophilus influenzae* type b (Hib) are also included in the programme (Table 1).

Table 1. Diseases, year of introduction and vaccine included in the Dutch National Immunisation Programme

Disease	Year of introduction in the National Immunisation Programme	Vaccine
Diphtheria	1952	DTP, since 1962 DTP-IPV
Tetanus	1952	DTP, since 1962 DTP-IPV
Pertussis	1952	DTP, since 1962 DTP-IPV
Poliomyelitis	1957	IPV, since 1962 DTP-IPV
Rubella	1974 (for girls)	R, since 1987 MMR
Measles	1976	M, since 1987 MMR
Mumps	1987	MMR
Haemophilus influenzae type b	1993	Hib

The vaccination schedule till 1999 is given in Table 2. Since January 1999, children are offered the first three vaccinations for DTP-IPV and *Haemophilus influenzae* type b at 2, 3 and 4 months of age instead of 3, 4 and 5 months of age (16). Advancing the programme will protect children earlier in life, which is particularly relevant to the prevention of pertussis and invasive *H. influenzae* type b infections.

*Table 2. Vaccination schedule of the Dutch National Immunisation Programme till January 1999**

Vaccine	Primary course (in months)	Booster vaccination (in years)
DTP-IPV, Hib	3, 4, 5 and 11	
DT-IPV		4 and 9
MMR	14	9

* Since January 1999 the schedule for the first three vaccinations has been accelerated by 1 month, starting at 2 months of age

The vaccination coverage amounts to 97% for all 12-month-old children for at least three vaccinations against DT(P)-IPV and 94% of all 2-year-old children for one vaccination against MMR (17). The high coverage is a result of a very effective organisation of vaccination in the Netherlands (18,19). Every child is offered vaccination from birth to 13 years on a voluntary basis and free of charge. The Dutch National Immunisation Programme is implemented mainly by the network of Maternal and Child Health Clinics for children up to the age of 4 years and by Public Health Services for school-aged children. The provincial immunisation administrations each maintain a database of vaccination records for each child younger than 13 years of age living in the province and process data on births, deaths and removal from municipal population records (18,19). Parents receive an invitation for vaccination with information on the importance of vaccination. If the provincial immunisation administration has not received a notice that the child has been vaccinated within a specified time, a reminder is sent.

Despite the average high vaccination coverage, in some municipalities or villages the vaccination coverage is less than 90%, sometimes even only 60% (17). These municipalities are in a belt from the southwest to the northeast of the country. There are several communities in these municipalities who refuse vaccination on religious grounds. These pockets of unvaccinated orthodox reformed persons are particularly important because they form a closely knit sociogeographical network and therefore could escape herd immunity (10,18,20). The remaining unvaccinated groups live scattered all over the country and appear to have various reasons for not being vaccinated, such as ignorance of the health system, socioethnic problems in immigrant populations and 'illegal' residency (17,18,21). A small group of anthroposophically oriented people resists immunisation, especially against measles, mumps and rubella, because of nature-based attitudes towards life (18,21).

Evaluation of the Dutch National Immunisation Programme: seroepidemiology of diphtheria, tetanus, poliomyelitis and pertussis

Before the licensing of a vaccine, the safety and efficacy of the vaccine must be established in controlled trials (22,23). However, post-licensing evaluation is of equal importance for the success of a vaccination programme. Epidemiological methods play an important role in this continuous evaluation to assess the safety of the vaccine, vaccination coverage, disease incidence, disease severity and the immunity of the population (22,23).

A passive surveillance system for monitoring adverse events following vaccinations, with a 24-hour telephone service, was instigated in the Netherlands in 1962 (24-26). In 1994, 712 adverse events were reported with about 2 million vaccinations administered. The interval between

vaccination and the event is established and the likelihood of causality with the administered vaccine is assessed for all reported adverse events (24).
The vaccination coverage in the Netherlands is reported annually by the Inspectorate of Health (17). As already stated, this coverage is consistently high with the exception of the groups mentioned.

The reduction in disease incidence achieved after a vaccine has been introduced into routine use is the best measure of success of a programme. In the Netherlands, most information on the incidence of vaccine-preventable disease is derived from notifications, laboratory reports, data on hospital admissions and registration of deaths (27). These data show that the incidence of vaccine-preventable diseases and their complications decreased considerably after the introduction of vaccination (27).
Since this thesis covers studies on diphtheria, tetanus, poliomyelitis and pertussis, the numbers of reported cases of these diseases are given in Figures 1 to 4. The numbers of notifications of diphtheria, tetanus and poliomyelitis in recent years are very small, while pertussis is endemic in the Netherlands and epidemics occur. Pertussis surveillance is useful for studying trends and detecting epidemics; it could possibly help identify factors involved in disease occurrence.
The small numbers of diphtheria, tetanus and poliomyelitis cases certainly do not mean that surveillance should be omitted for these diseases. For example, in view of the initiative of the World Health Organization to eradicate poliomyelitis, countries should detect at least 1 case of nonpolio acute flaccid paralysis per 100,000 inhabitants to confirm the absence of poliomyelitis. This nonpolio acute flaccid paralysis rate is a measure of the sensitivity of the surveillance, or, in other words, the ability of a country's surveillance system to detect a poliomyelitis case if it were to occur in the population (28).
However, with smaller numbers of cases, the role of serological surveillance as an epidemiological method for the evaluation of the National Immunisation Programme becomes more evident. Immunisation programmes aim to replace naturally acquired immunity by vaccine-induced immunity. Neither history of disease nor vaccination is necessarily an accurate marker of true immunity (22). Therefore, if a serological correlate of protective immunity against a vaccine-preventable disease exists, serological surveys are useful. Assessment of specific antibodies offers the opportunity to identify groups with little immunity that might require changes in vaccination strategy to prevent (re)emerge of the disease (22,29). In order to make reliable estimates of the immunity of the population, the sample of sera used for serosurveillance should be representative of the total population. A population-based serum collection with extensive information on study subjects would be ideal. A rare example is the National Health and Nutrition Examination Study (NHANES) in the United States (30).

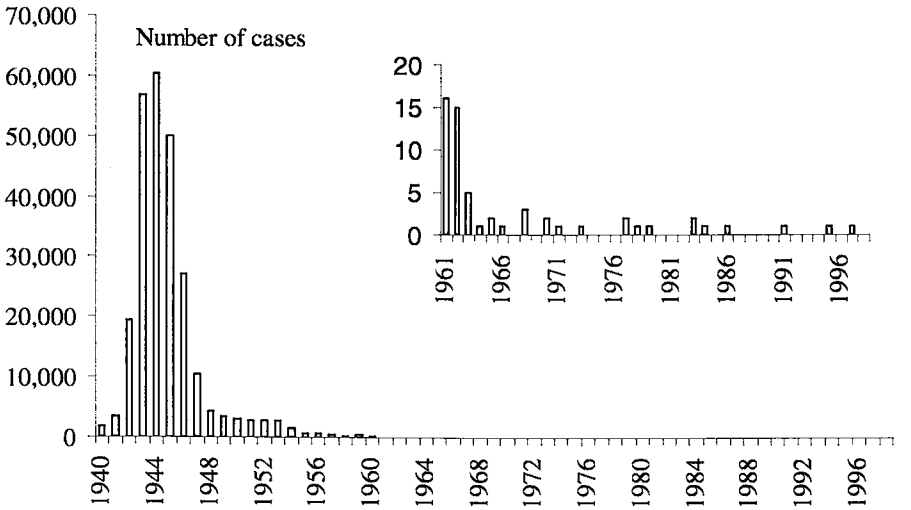

Figure 1. Numbers of diphtheria cases reported from 1940 to 1998.

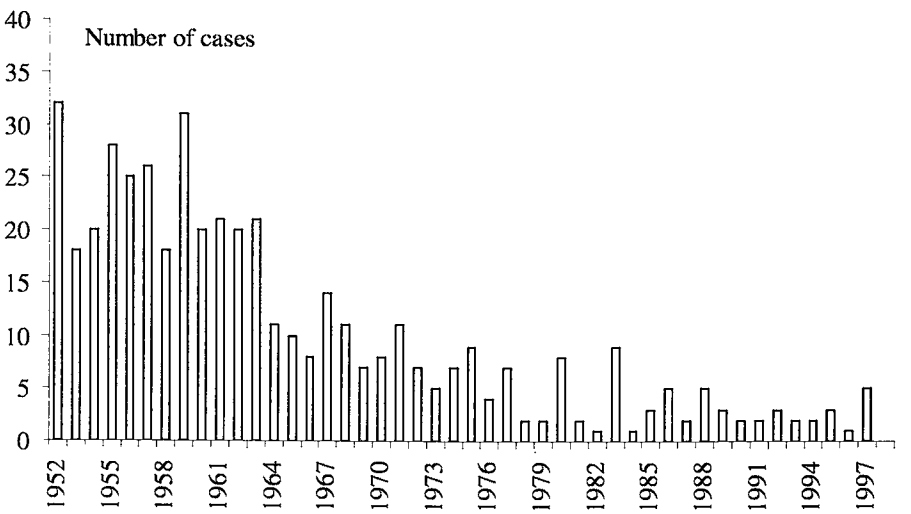

Figure 2. Numbers of tetanus cases reported from 1952 to 1998.

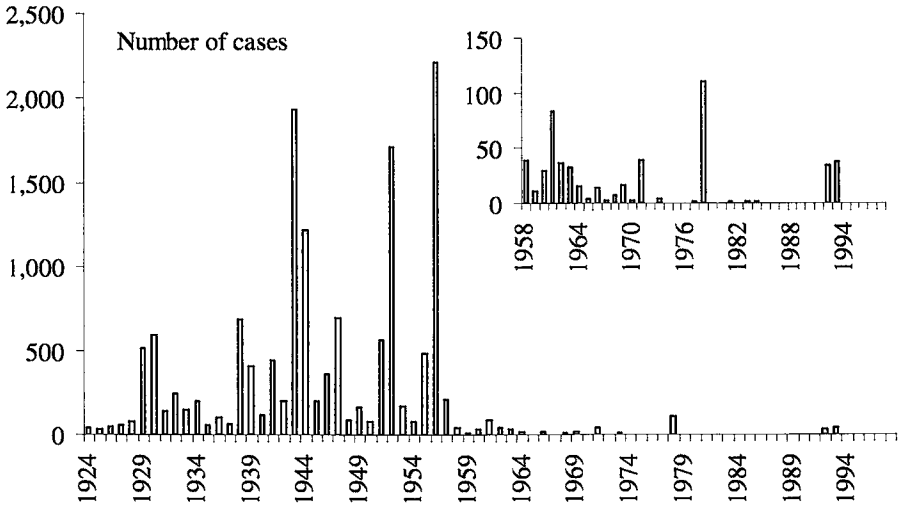

Figure 3. Numbers of poliomyelitis cases reported from 1924 in 1998.

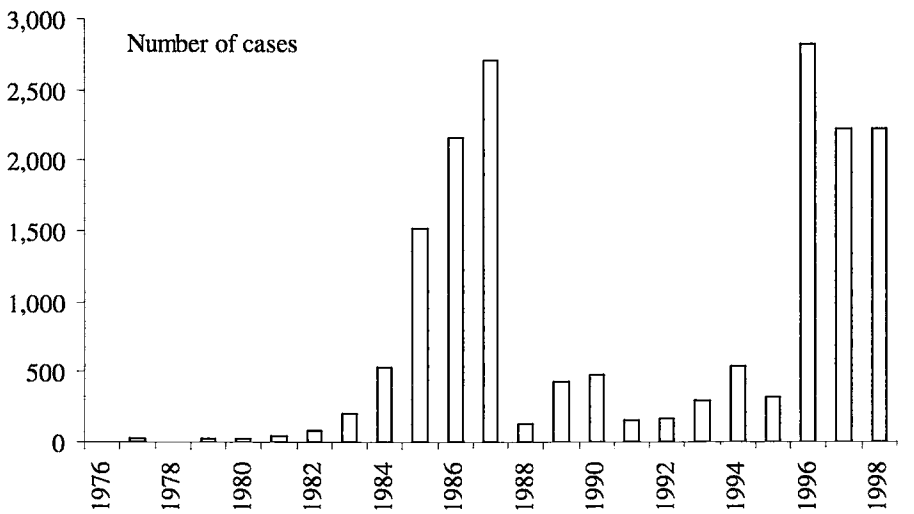

Figure 4. Numbers of pertussis cases reported from 1976 to 1998.

Antibodies that correlate with protection are known for diphtheria, tetanus and poliomyelitis, but not for pertussis (31-36). Thus, for diphtheria, tetanus and poliomyelitis, serological surveillance is a useful tool for providing insight into the long-term effects of mass vaccination. Serological data for pertussis are more difficult to interpret regarding population protection. However, serological data on pertussis give valuable information on the antibody distribution in the general (healthy) population, which can be used to improve the serodiagnosis of pertussis. Laboratory methods are required to confirm the clinical diagnosis of pertussis. Since pertussis diagnosis is hampered by low sensitivity, improvement of the diagnostic tools are beneficial for both the individual patient and for disease surveillance (37).

Outline of the thesis

In view of the evaluation of the Dutch National Immunisation Programme, the main objective of the studies described in this thesis was to provide insight into the immunity to diphtheria, tetanus and poliomyelitis and into the occurrence of pertussis in the Netherlands and to improve the serodiagnosis of pertussis.

This thesis is based mainly on two studies. In the first study, a population-based serum bank was established by collecting sera from the general population together with questionnaire data on infectious disease determinants. This nationwide study includes individuals from the general population in all age groups from 0 to 79 years. Furthermore, sera were also collected from the general population in municipalities with a lower vaccine coverage. Differences in immune status of individuals from the nationwide sample and the latter sample are of particular interest in the Netherlands with its specific unvaccinated groups.
In the second study data on the number of pertussis patients from various surveillance sources such as notifications, serodiagnostic data and hospital admissions, were analysed.

This thesis consists of three parts. The first part is entitled "A population-based serum bank". **Chapter 2** describes the role of serological surveillance as an epidemiological method in the evaluation of a national immunisation programme and the design of the population-based serum bank. **Chapter 3** described the results of the nonparticipation study that is a part of the first study. In the second part of the thesis, entitled "Immunosurveillance for diphtheria, tetanus and poliomyelitis", the prevalence of diphtheria antitoxin antibodies (**Chapter 4**), of tetanus antitoxin antibodies (**Chapter 5**) and of neutralising antibodies against poliovirus types 1, 2 and 3 (**Chapter 6**) are studied with respect to the Dutch general population as well as to orthodox reformed groups who refuse vaccination in the municipalities with low vaccine coverage.
The last part of the thesis is entitled "Surveillance and seroepidemiology of pertussis". **Chapters 7 and 8** describe surveillance data of pertussis, which were studied to obtain insight into the

pertussis epidemic in 1996 and 1997 and to find possible explanations for this outbreak. **Chapter 9** investigates whether high levels of IgG antibodies against pertussis toxin in a single serum sample provide a specific laboratory tool for the diagnosis of pertussis. Finally, in the general discussion (**Chapter 10**), the main findings of the studies and possible implications for public health and further research are discussed.

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Part 1

A population-based serum bank

Chapter 2

Immunosurveillance and the evaluation of national immunisation programmes: a population-based approach

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Abstract

Mass vaccination can change the epidemiological dynamics of infectious diseases. It may result in a limited persistence of natural and vaccine-induced immunity and a higher mean age of infection, which may lead to a greater risk of complications. The epidemiological situation should be monitored and immunosurveillance based on the assessment of specific antibodies against vaccine-preventable diseases in human serum is one of the tools. In order to estimate the immunity of the Dutch population reliably, a large-scale, population-based, collection of serum samples was established (8359 sera in a nationwide sampling and 1589 sera from municipalities with low vaccine coverage). In contrast to collecting residual sera from laboratories, this approach gains extensive information by means of a questionnaire regarding the determinants of the immune status and the risk factors for the transmission of infectious diseases in general. The population-based approach gives a better guarantee that the data are representative than collecting sera from laboratories does.

Introduction

In the Netherlands, we have established a bank of sera from the general population for public health research. This article describes the use of seroprevalence data for the evaluation of the National Immunisation Programme (NIP), the design used for the data collection and the advantages of a population-based approach. Such an approach may be useful for future sero-epidemiological studies in other countries. Results drawn from seroprevalence data from this study on vaccine-preventable diseases will be published separately.

Long-term epidemiological effects of mass immunisation

As in other countries, the incidence of most vaccine-preventable diseases (and their complications) in the Netherlands decreased considerably after the introduction of immunisation with diphtheria, tetanus and pertussis (DTP) vaccine in 1952, inactivated polio vaccine (IPV) in 1957, rubella vaccine for girls in 1974, measles vaccine in 1976, measles, mumps and rubella (MMR) combination vaccine in 1987 and *Haemophilus influenzae* type b vaccine in 1993. The vaccine coverage is high and amounts to 97% of all 12-month-old children for three immunisations against DTP-IPV and 94% of all 14-month-old children for one immunisation against MMR (1).

However, despite this high vaccine coverage, epidemics still occur (e.g measles and pertussis), and the (re)emergence of diphtheria is possible (2-5). Furthermore, mass vaccination may have some secondary effects in the longterm, as the epidemiological dynamics of infectious diseases can change. Once a pathogen has been pushed back to a large extent and its circulation is limited, the force of infection (the rate at which those who are susceptible acquire infection) decreases (6). This means that the chance of infection will decrease, which will result in delaying the infection of susceptible (unvaccinated) individuals. The expected increase in mean age for some vaccine-preventable diseases is related to a greater chance of complications. For example, the frequency of orchitis due to mumps infection increases with age, the case fatality rate for measles was highest for unvaccinated individuals in older age groups and the shift in rubella infections to childbearing age could result in a greater risk of congenital rubella syndrome (CRS) (7-9).

The lower force of infection also results in lack of boosting opportunities of both natural and vaccine-induced immunity. In contrast to the past, natural immunity may not persist lifelong, and vaccine-induced immunity may be lost even faster (10,11). This may also result in a shorter duration of passive immunity due to maternal antibodies in infants (12). Therefore, in order to assess the long-term effects of mass immunisation, insight into the (possibly changing) duration of vaccine-induced immunity and natural immunity is necessary.

Herd immunity implies that non-immune individuals are protected from infection by the presence of immune individuals (13). Thus, in order to prevent the further spread of an infection, a

proportion of immune individuals in the population below 100% is sufficient. The threshold value is dependent on the contact rate between individuals and the probability of transmission. This was estimated (homogenous mixing assumed) at 82%-87% for diphtheria, poliomyelitis and rubella, at 85%-90% for mumps and at 90%-95% for measles and whooping cough (6). Nevertheless, even in countries with high vaccine coverage, infections are still a threat, since high coverage on the average does not, warrant sufficient herd immunity. For example, in the Netherlands, groups who reject vaccination on religious ground, are sociodemographically and geographically clustered. In such situations where the condition of homogeneous mixing is not fulfilled, herd immunity can break down and epidemics can occur as a result. Such an event took place during the polio epidemic in 1992-3 (14). This epidemic was restricted to religious groups in a closely knit social network. The high incidence of CRS after a rubella epidemic among the Amish people in the United States is another example of insufficient herd immunity. Due to the low vaccine coverage and social clustering in combination with the absence of regular contacts outside their own community, the number of susceptible individuals had increased (8).
Potential long-term secondary effects of mass immunisation should be anticipated by surveillance and possible adaptations of vaccination policy should be considered.

Evaluation of immunisation programmes from seroprevalence data

Epidemiological methods play an important role in the evaluation of an immunisation programme. These methods include monitoring vaccine coverage, surveillance of the occurrence of vaccine-preventable diseases, case investigations, outbreak investigations, vaccine efficacy and effectiveness studies, surveillance of vaccine safety and serological surveillance (15,16).
Important information on vaccine-preventable diseases can be derived from the latter method known as serosurveillance, which is the assessment of specific antibodies in serum as a sign of previous contact with the pathogen (17). These antibodies could have been induced either by natural infection with the virulent pathogen or by immunisation with the inactivated or live, attenuated pathogen. However, in general no serological methods are yet available to distinguish between natural and vaccine-induced immunity for vaccine-preventable diseases. Nevertheless, for some diseases the distinction can be made indirectly. Combining anti-Hbs and anti-Hbc tests the presence of the former in absence of the latter indicates vaccine-induced immunity against hepatitis B virus. Poliovirus specific IgA antibodies seem to discriminate between natural or OPV-induced and IPV-derived immunity (18,19). For most vaccine-preventable diseases, specific antibodies can be used as an indicator of protection against the disease (for instance the presence of neutralizing antibodies against poliomyelitis).
Antibodies induced by natural infection give information about both clinical and subclinical infections. Thus, this surveillance source is not limited to individuals with diseases that are otherwise considerably underreported (20-23). For example, evidence was found of subclinical

pertussis infections in which one-fifth of the youngest children had antibodies to pertussis toxin, even though they had no history of whooping cough (24).

Serosurveillance offers an opportunity to look for clustering of susceptible individuals within specific age, social or geographical groups based on serological profiles. Ideally, identifying these groups should result in preventive interventions (for example, efforts to increase vaccine coverage in such groups or to adapt the vaccination strategy) before more cases (re)emerge. Low antibody levels for diphtheria and tetanus found in adults stress the need for revaccination of adults (25-28). Seroprevalence studies support the two-dose immunisation strategy for MMR to prevent the disease from shifting to older age groups (29-31).

Seroprevalence data can also benefit modelling studies (6). On the one hand, seroprevalence data derived from serological surveys can be used to estimate input parameters in modelling studies. The dynamics of the disease occurrence can then be predicted. For example, the data can help estimate the force of infection, the average age of infection and the minimum proportion of immune individuals needed to prevent transmission (the herd-immunity threshold). On the other hand, serological data are important in modelling studies to test the accuracy of the model predictions and epidemiological assumptions. Differences in the predicted serological profile and the observed serological profile may indicate the (in)correctness of the model assumptions (9,32-34). Results from serological surveillance in England reveal that the proportion of school children susceptible to measles was increasing after the introduction of the MMR vaccination programme in 1988. Mathematical models were used to interpret these data. As the models predicted an epidemic of measles in 1994, a national campaign for measles and rubella vaccination was held in the United Kingdom (9). In the Netherlands in 1987, the selective vaccination strategy against rubella and CRS was changed to a mass vaccination strategy with MMR vaccine. Seroprevalence data were used as an input parameter in the mathematical models. These models played a major role in the decision-making process for changing the strategy (35).

In order to make reliable estimates of the immunity of the population, the sample of sera used for serosurveillance should be representative for the total population in age and sex structure. The sample should also be representative for other demographic and socio-economic factors. Therefore, ideally, a population-based survey should be repeated periodically to study any changes in the population immunity. Sera from blood banks, military recruits or specialist clinics are biased towards certain age groups and sections of the community. Many serosurveys use serum samples from the more readily accessible sources (26,27,30,31,36-43). Although these studies are important in monitoring the effects of immunisation programmes, they may lack or be representative to an unknown degree. Stark and colleagues suggest that immunity against diphtheria in blood donors might lead to an overestimation of the immunity in the population, as blood donors might be more health conscious than the average individual (26). Whether individuals attending special clinics are more or less likely to have antibodies for a specific

pathogen than those in the general population is unknown. Apart from not being representative, another disadvantage is that the information about individuals from whom such sera were derived is often limited to age, sex and sometimes vaccination history.
Serosurveys based on random samples are described, but often limited to a small age range or specific region or city (24,44-46). The National Health and Nutrition Examination Study (NHANES) in the United States is a rare example with a population-based serum collection and with extensive information on study subjects (25).
In the Netherlands we have also had the opportunity to collect sera from the general population together with questionnaire data on determinants. This nationwide study includes individuals from the general population in all age groups from 0-79 years. Furthermore, sera were also collected from the general population in municipalities with the lowest vaccine coverage. Differences in immune status of individuals from the nationwide sample and the latter sample are of particular interest in the Netherlands with its specific unvaccinated groups. The data can give some insight into the effects of clustering of unvaccinated individuals on herd immunity.

A population-based collection of serum samples in the Netherlands

Sampling

A two-stage cluster sampling technique was used to draw a nationwide sample. In each of five geographic regions, with approximately equal numbers of inhabitants, eight municipalities were sampled proportionally to their size. Within each municipality, an age-stratified sample of 380 individuals was drawn from the population register. The population register contains all individuals with a home or postal address. Homeless without a postal address and illegal aliens are not included in the register. The age strata were 0, 1-4, 5-9, ... 75-79 years. In each of the first two strata 40 individuals were sampled, while in each of the following strata 20 individuals were sampled. This oversampling was based on an expected lower response (25% instead of 50%) from very young children and the importance of sufficient data in these age groups. Otherwise, we could not obtain insight into the level of maternal antibodies and the mean age of infection.
Because of the particular situation in the Netherlands with its geographically clustered groups who refuse immunisation, records from the population registers of eight municipalities with a consistently low(er) immunisation coverage were also sampled. The vaccine coverage in these municipalities for three DTP-IPV immunisations of 12-month-old children ranged from 65-87% in 1995 (1). The objective was to gain access to more unvaccinated individuals and to obtain more accurate estimates of seroprevalence in this subgroup. The expected number of unvaccinated individuals in the national sample would be too small to estimate seroprevalence for the diseases in the NIP in this subgroup.

The number of clusters (municipalities) and units (individuals) per cluster were chosen so that the expected accuracy of the seroprevalence estimates would be optimal within financial and logistical constraints. The accuracy is determined mainly by the total number of clusters and to a lesser extent by the number of units per cluster, as the expected variance between clusters is greater than within clusters (47).
In total, 18 217 individuals were invited: 15 189 in the national sample and 3028 in the sample of the municipalities with a low vaccine coverage.

Data collection

The data were collected from October 1995 to December 1996 in collaboration with the public health services, an organization well known to the local inhabitants. The prospective participants were approached by mail and were asked to fill in a questionnaire at home and to visit the special clinic to give a blood sample (20 ml). The questionnaire asked for data on gender, occupation, level of education, country of birth and nationality, participation in military service, vaccination (participation in the NIP, opinion on the necessity of vaccination against DTP-IPV, MMR and Hib, (re)vaccination against DTP, tetanus, Hib, hepatitis A, hepatitis B, influenza), religion, travel, long-term coughing, pertussis, otitis, diabetes, gardening, contact/keeping animals, recreation in fresh waters, sexually transmitted diseases, self-perception of health, chronic diseases, smoking and drinking habits. Participants were asked to bring their immunisation certificates. In a pilot study in 1994, it turned out that self-reported vaccination history was not reliable (48). Therefore the analysis of serological data to investigate waning immunity will be directed toward individuals with verified vaccination history.
An invited individual received a letter of invitation, along with a brochure giving information on the study, a questionnaire and a prescheduled appointment time (between 09.00 and 17.00 h). If the suggested time was not convenient, the participant could choose another time during the 2 days when the clinic was held in the municipality. The walk-in clinic was open from 17.00 to 19.30. An extra clinic appointment could be made 1 week later, or a house-visit could be arranged.
Turkish and Moroccan residents received a letter of invitation in their own language. They were told that a Turkish and Moroccan speaking nurse would be present. Special attention was given to these nationalities because we expected a lower response rate, considering the results of the pilot study. These nationalities were important as the seroprevalence might be different. There might be a different force of infection and a different vaccination policy in their countries of birth. Access to the NIP and healthy baby clinics could be limited by language problems and frequent change of address for those who had not been in the Netherlands long. Before the consultation days, we telephoned invited individuals to remind them of the study, to answer any questions and to ask if they were willing to participate. In the pilot study, it was shown that the approach by telephone

led to a 6% increase in the response rate (49). When individuals declined to participate, they were asked to fill in the questionnaire or at least to answer some questions for the non-response survey (by telephone or mail). Individuals who could not be reached by phone were sent a written reminder. Participants were offered a gift voucher. Participants had to sign a written informed consent form that stated that the sera were to be tested for specific antibodies against infectious diseases (with the exception of HIV), that the data would be processed anonymously, that the serum would be coded and stored for a long time for the purpose of public health research and that they would not be informed of the test results. The study proposal was approved by the Medical Ethical Committee of Netherlands Organisation for Applied Scientific Research (TNO), Leiden, The Netherlands.

Non-response survey

Information about the participants regarding age, gender, marital status and nationality was available from the population registers. In addition, individuals who declined to participate were asked to fill in the original questionnaire or a short non-response questionnaire that contained questions about their level of education, religion, participation in the NIP, opinion on vaccination against diseases in the NIP and self-perception of health. The information about nonparticipants offers us the opportunity of correcting the seroprevalence data for possible selective nonparticipation.

Serum processing and storage

The blood samples were stored in a refrigerator during the day and at night. The sera were harvested the next day and divided into portions of 350 µl which were stored at minus 86 °C in different freezers.
The methods used are described in more detail in reference number 50.

The serum bank

A serum bank of 9948 samples has been established. All public health services and municipalities co-operated in the study. The (non)response rates for both samples are given in Table 1.
The adjustments (reminder before consultation hours, Moroccan and Turkish speaking nurses, translated letter of invitation to individuals with Moroccan or Turkish nationality) made on the basis of the findings of the pilot study seem to have been successful because the participation rate increased from 40% in the pilot study to 55% (49). The participation rate was only slightly lower in the sample of municipalities with a low vaccine coverage (52.5%).

Table 1. (Non)response rates in the nationwide and low vaccine coverage sample, Pienter project 1995-6, The Netherlands

	Nationwide sample		Low vaccine coverage sample	
	N	(%)	N	(%)
Participants				
Questionnaire and blood sample	8359	(55.0)	1589	(52.5)
Nonparticipants				
Full questionnaire	1618	(10.7)	375	(12.4)
Nonresponse questionnaire	1053	(6.9)	187	(6.2)
Information from the population register	4159	(27.4)	877	(29.0)
Total invited	15189	(100)	3028	(100)

The serum bank can facilitate many sero-epidemiological studies. It will mainly be used for vaccine-preventable diseases, but the serum bank can also be used to obtain insight into the occurrence of infectious diseases with a course that is frequently subclinical and into the prevalence of other determinants. A procedure has been set up for release of the sera for further research. Research proposals will be judged on the relevance for public health and on scientific quality by a team of experts.

Seroprevalence studies for diphtheria, tetanus, poliomyelitis, mumps, measles, rubella, *Haemophilus influenzae* type b, hepatitis A, B and C are currently in progress.

Conclusions

It appeared feasible to establish a serum bank in the Netherlands for public health research through a large-scale nationwide population-based cross-sectional study of the general population. The data will primarily be used for the evaluation of the effects of the NIP. Therefore, following an identical sampling scheme, a parallel study was done in municipalities with low-vaccine coverage. These municipalities are of particular interest in our country, with its specific religious groups who refuse vaccination. A response rate of 55% was achieved. The result was a collection of nearly 10 000 sera. In contrast to the collection of residual sera from laboratories, this approach allowed for the collection of extensive information by means of a questionnaire regarding determinants of the immune status and risk factors for transmission of infectious diseases in general. An advantage of the population-based approach is that it gives a better guarantee that the data are representative. Besides, the information on non-participants offers an opportunity to study the impact of non-participation on seroprevalence estimates. In order to monitor changes in the population's immunity the serosurvey should be repeated periodically,

perhaps every 5 to 10 years, depending on the postulated rate of loss of immunity. It could also be considered to build up the serum bank in a more continuous way by collecting sera every year. This allows for the exploration of the effects of intercurrent epidemics (pertussis in 1996/7 or poliomyelitis in 1992/3 in our country) and either the introduction of new vaccines or changes in the (schedule of the) vaccines applied routinely (5,14). In the Netherlands we are now moving to such a continuous population-based serum collection. Although it would be even more interesting to include a longitudinal component in the survey it seems up to now not feasible since it would increase the already high costs of a population-based survey enormously.

Despite the high costs of this population-based study compared to the use of residual sera, it might be worthwhile for other countries to consider a population-based collection of serum samples to study immunity against vaccine-preventable diseases. In 1996, the European Sero-Epidemiology Network (ESEN) was established to co-ordinate and harmonise the serosurveillance of immunity to vaccine-preventable diseases in six European countries (Denmark, England, France, Germany, Italy and the Netherlands) (51). Laboratory methods should be standardized so that results from each centre are directly comparable. This is one of the major aims of the ESEN. However, comparability is also dependent on the representativeness of the study populations. In the ESEN, most of the countries had to rely on specimens submitted to laboratories for diagnostic purposes. Actually, not only should serological methods be standardised, the serum collection should be standardised as well, preferably by random sampling as advocated in this article.

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Chapter 3

Nonparticipation in a population-based seroprevalence study of vaccine-preventable diseases

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Abstract

To estimate the immunity of the Dutch population against vaccine-preventable diseases, an population-based serum bank was established. Since a multi-tiered approach to enrol eligible individuals was used, both the overall nonresponse selection and the effect on this selection of including additional participants and of excluding a subgroup of nonparticipants (i.e. those without questionnaire data) could be studied. For some characteristics associated with nonparticipation, an association with seroprevalence of vaccine-preventable diseases is likely (e.g. age, gender). For other characteristics (e.g. marital status, reminder, degree of urbanisation) the association with immune status is unclear but probably small. If the population's distribution or information on all participants and nonparticipants of the characteristic is available, the effect on the seroprevalence can be estimated. However, investigators have to be aware that studying only a subgroup of nonparticipants might lead to biased insight into nonparticipation selection. Furthermore, including additional participants might not always reduce nonparticipation selection.

Introduction

To estimate the immunity of the Dutch population against vaccine-preventable diseases, we established a serum bank of Dutch individuals in a population-based study (1). Most previous serosurveys used residual sera from blood banks, military recruits, or specialist clinics which are not representative. Despite an appropriate sampling frame, serum collection may still be biased due to selective nonparticipation (2-8). To reduce this bias, one may incorporate information on characteristics of nonparticipants into sample estimates (9). In our study, a multi-tiered approach to enrol eligible individuals was used. This approach made it possible to study not only the overall nonresponse selection in our serum collection which will be used for future seroprevalence studies, but also to study the effect of including additional participants and of excluding a subgroup of nonparticipants on this selection (10-12).

Material and methods

Sampling design

In each of five geographic regions in the Netherlands with similar population sizes, eight municipalities were sampled with a probability proportional to their population size. An age-stratified sample of approximately 380 individuals was randomly selected within each municipality from the municipal vital statistics information (1). The age strata were 0, 1-4, and then by 5-year classes up to 75-79 years. Twenty individuals were sampled in each stratum except the first two, where 40 individuals were sampled as a lower response rate was observed in these age groups in a pilot study (13). Data were collected between October 1995 and December 1996. Subjects were contacted by mail and requested to fill out a questionnaire and to visit a clinic for blood sampling on one of two preset dates during daytime or evening hours. Before these dates, individuals were reminded of the study by telephone, if possible, or in writing. Individuals who were unable to attend on either of the two preset dates could visit an extra clinic. Those who refused to participate were asked to fill out the full self-administered questionnaire or, failing that, a short nonresponse questionnaire.

Five groups of (non)participants were distinguished:

1. 7,904 (52.0%) "initial participants" gave blood at the regular clinic and completed the questionnaire.
2. 455 (3.0%) "additional participants" gave blood at the extra clinic and completed the questionnaire.
3. 1,618 (10.7%) nonparticipants only completed the full questionnaire.
4. 1,053 (6.9%) nonparticipants only completed the nonresponse questionnaire.

5. 4,159 (27.4%) "absolute nonparticipants" neither gave blood nor completed a questionnaire.

For all groups, data on an individual level were available on age, gender, marital status (with the exception of one municipality), nationality (with the exception of two municipalities) from the municipal database; municipality and degree of urbanization of the municipality were assigned based on the place of residence of the individuals ("nonquestionnaire" variables). Missing data on nationality and/or marital status were imputed with data from other municipalities. Information on educational level, self-perception of health, religion, and opinion of the importance of immunisation were collected with both questionnaires ("questionnaire" variables).

Definition of variables and statistical analysis

The 17 age strata were grouped into five classes: 0-4, 5-14, 15-29, 30-64, and 65-79 years, which corresponded well with participation rates. Marital status was classified as "married", "unmarried", and "widowed or divorced". Nationality was classified as "Dutch", "Turkish", "Moroccan", or "other". Although the numbers of Turks and Moroccans were small, they were analysed separately as a special effort was made to improve their response. Self-perception of health was classified as "(very) good" and "less than good" and reminder status as "by telephone", "by mail", or "other" (i.e., no reminder or unknown). The following categories for degree of urbanization were made: "very high" (>2,500 addresses/km^2), "high" (1,500-2,500), "moderate" (1,000-1,500), "low" (500-1,000), and "none" (< 500). The geographical regions were based on the provinces in the Netherlands: "Central" (Utrecht and Gelderland), "Southeast" (Brabant and Limburg), "Northwest" (Noord-Holland and Flevoland), "Southwest" (Zeeland and Zuid-Holland) and "Northeast" (Groningen, Drente, Overijssel, and Friesland).
The highest educational level achieved by those aged 17 years or older and by one of the parents for those younger than 17 years were classified as "low" (primary school, lower vocational or lower general secondary education), "intermediate" (intermediate vocational or intermediate general secondary and higher general secondary education), and "high" (higher vocational secondary education and university education). Three religious groups were distinguished: "Orthodox Reformed" for those who are known to be opposed to vaccination, "Reformed Bond" of whom about a quarter reject vaccination, and "other or no religion" (14).

For the opinion of "importance of immunisation" the following groups were distinguished:

1. Diphtheria (D), pertussis (P), tetanus (T), poliomyelitis (IPV), *Haemophilus influenzae* type b (Hib), mumps (M), measles (Me), and rubella (R) immunisation were considered necessary.
2. Seven of the eight immunisations just mentioned were considered necessary.

3. All immunisation was considered unnecessary.
4. D,T,P,IPV, and Hib were thought necessary and M,Me, and R unnecessary.
5. M,Me,R were thought necessary and D,T,P,IPV, and Hib "otherwise" (less than four necessary)
6. D,T,P,IPV, and Hib were considered necessary and M,Me,R "otherwise" (less than two necessary).
7. The remaining group had some other opinion.

Although the number of subjects in the categories "D,T,P,IPV,Hib,M,Me, and R unnecessary" and "Orthodox Reformed" were small, these were considered separately because these groups decline immunisation often, which might affect our evaluation of the national immunisation programme.

All participants were compared to all nonparticipants in the first dichotomous logistic regression and "non-questionnaire" variables that were associated with (non)participation were identified. Variables remained in the multivariate model if either the likelihood ratio test was significant ($p < 0.05$) or the estimates of the beta coefficients for other variables in the model changed by at least ten percent. These variables were included in the polytomous logistic regression model.
The second dichotomous logistic regression analysis was restricted to (non)participant groups with questionnaire data. "Nonquestionnaire" variables from the first dichotomous logistic model were included. "Questionnaire" variables were included in the dichotomous and polytomous models according to the procedure described. Participants were used as reference group in the dichotomous logistic models; initial participants, in the polytomous logistic models.

Results

The univariate and mulitivariate analyses showed that several subgroups were less likely to participate: men aged 15-29 and 30-64 years versus women in these age groups, girls or women aged 0-4, 15-29, or 65-79 years versus women aged 30-64 years, boys or men aged 0-4 and 15-29 years versus men aged 30-64 years, women aged 15-29 versus men aged 15-29 years and women aged 30-64 versus men aged 30-64 years, unmarried or widowed/divorced versus married individuals, individuals with Turkish or "other" nationality versus Dutch individuals, individuals who did not receive a reminder by telephone versus those who did and individuals from municipalities with (very) high or moderate versus no degree of urbanization (Table 1). More likely to participate were: boys aged 5-14 versus men of 30-64 years, girls aged 5-14 years versus women aged 30-64 years, Moroccans versus Dutch, and individuals from the southeast versus northeast region (Table 1). The likelihood of participation was similar for men and women aged 0-4, 5-14 and 65-79 years.

Table 1. Odds ratios (ORs) and 95 percent confidence intervals (95% CI) for characteristics ("Nonquestionnaire" variables) comparing all nonparticipants with participants; Pienter Project, 1995-1996, The Netherlands

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
Age group (years) by gender					
Male					
0-4	1,636	1.34	(1.18 - 1.51)	1.00	(0.85 - 1.18)
5-14	820	0.48	(0.40 - 0.56)	0.36	(0.29 - 0.44)
15-29	1,194	1.65	(1.44 - 1.89)	1.35	(1.14 - 1.59)
30-64*	2,842	1.0		1.0	
65-79	1,017	1.01	(0.88 - 1.17)	1.06	(0.92 - 1.23)
Female					
0-4	1,551	2.06	(1.82 - 2.34)	1.62	(1.37 - 1.91)
5-14	785	0.78	(0.66 - 0.93)	0.58	(0.47 - 0.71)
15-29	1,205	1.47	(1.28 - 1.68)	1.22	(1.03 - 1.43)
30-64*,†	2,756	1.0		1.0	
65-79	1,383	2.02	(1.78 - 2.31)	1.88	(1.64 - 2.16)
Marital status					
Married*	6,012	1.0		1.0	
Unmarried	7,769	1.42	(1.33 - 1.52)	1.44	(1.27 - 1.63)
Widowed/divorced	1,408	1.86	(1.66 - 2.09)	1.59	(1.40 - 1.80)
Country of nationality					
Netherlands*	14,568	1.0		1.0	
Turkey	128	1.33	(0.94 - 1.89)	1.12	(0.78 - 1.61)
Morocco	128	0.80	(0.56 - 1.15)	0.65	(0.45 - 0.95)
Other	365	2.52	(2.03 - 3.15)	2.41	(1.91 - 3.03)
Reminder					
By telephone*	7,348	1.0		1.0	
By mail	7,386	1.38	(1.29 - 1.47)	1.31	(1.22 - 1.40)
Other	455	6.56	(5.16 - 8.35)	6.38	(4.99 - 8.15)

Table 1 continued

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
Degree of urbanization					
Very high	2,280	2.03	(1.83 - 2.25)	1.92	(1.72 - 2.15)
High	1,900	1.20	(1.07 - 1.34)	1.20	(1.06 - 1.35)
Moderate	3,798	1.07	(0.98 - 1.17)	1.10	(1.00 - 1.22)
Low	3,040	1.01	(0.92 - 1.11)	1.03	(0.92 - 1.14)
No*	4,171	1.0		1.0	
Region					
Central	3,040	0.98	(0.89 - 1.09)	0.93	(0.83 - 1.05)
Southeast	3,040	0.90	(0.81 - 1.00)	0.84	(0.76 - 0.94)
Northwest	3,038	1.06	(0.96 - 1.18)	1.00	(0.90 - 1.13)
Southwest	3,033	1.10	(0.99 - 1.21)	0.95	(0.84 - 1.07)
Northeast*	3,038	1.0		1.0	

* Reference group

† Women aged 30-64 years versus men aged 30-64 year crude OR 0.64 (95% CI 0.58 - 0.72) and adjusted OR 0.62 (95% CI 0.56 - 0.70)

The results from the multivariate polytomous logistic regression analysis (Table 2), were similar to those of the univariate analysis (not presented). For age groups by gender, the odds ratios for the various nonparticipant groups over initial participants were different. This was most obvious for the youngest age group: for both boys and girls the odds ratio for nonparticipants with full questionnaire over initial participants was far above unity, while it was below one comparing absolute nonparticipants with initial participants.

Nationality, kind of reminder, and degree of urbanization affected the types of (non)participation differently (Table 2).

To study the nonparticipation selection for "questionnaire" variables, absolute nonparticipants were excluded from the subsequent analysis. In the univariate analysis, educational level, religion, importance of immunisation, and self-perception of health were associated with nonparticipation (Table 3).

After adjustment for the "nonquestionnaire" variables, the opinion of the importance of immunisation on nonparticipation remained statistically significant. The polytomous logistic regression analysis shows that the opinions about the importance of immunisation affected the types of (non)participation differently (Table 4).

Table 2. Odds ratios (ORs) and 95 percent confidence intervals (95% CI) for characteristics ("Nonquestionnaire" variables) comparing additional participants (AP; n=455), nonparticipants with a full questionnaire (OQ; n=1,618), nonparticipants with a nonresponse questionnaire (NQ; n=1,053) and absolute nonparticipants (NP; n=4,159) with initial participants (IP); Pienter Project, 1995-1996, The Netherlands

	Adjusted ORs AP		Adjusted ORs OQ		Adjusted ORs NQ		Adjusted ORs NP	
Age group (years) by gender								
Male								
0-4	0.63	(0.39 - 1.01)	3.54	(2.62 - 4.78)	1.11	(0.81 - 1.52)	0.51	(0.42 - 0.62)
5-14	0.54	(0.32 - 0.91)	0.74	(0.50 - 1.09)	0.45	(0.30 - 0.68)	0.26	(0.20 - 0.33)
15-29	1.29	(0.83 - 2.02)	1.72	(1.23 - 2.41)	1.49	(1.08 - 2.06)	1.29	(1.07 - 1.56)
30-64*	1.0		1.0		1.0		1.0	
65-79	0.46	(0.27 - 0.78)	1.04	(0.76 - 1.41)	0.87	(0.63 - 1.20)	1.07	(0.91 - 1.26)
Female								
0-4	1.11	(0.69 - 1.78)	4.07	(3.01 - 5.49)	1.67	(1.21 - 2.32)	0.94	(0.77 - 1.16)
5-14	0.86	(0.51 - 1.46)	1.37	(0.97 - 1.95)	0.61	(0.40 - 0.93)	0.38	(0.29 - 0.49)
15-29	0.92	(0.58 - 1.48)	1.46	(1.07 - 2.01)	1.47	(1.07 - 2.02)	1.06	(0.88 - 1.29)
30-64*,†	1.0		1.0		1.0		1.0	
65-79	0.51	(0.30 - 0.88)	1.83	(1.42 - 2.37)	1.30	(0.96 - 1.77)	1.97	(1.68 - 2.31)
Marital status								
Married*	1.0		1.0		1.0		1.0	
Unmarried	1.33	(0.93 - 1.89)	1.33	(1.03 - 1.71)	1.55	(1.21 - 1.99)	1.46	(1.26 - 1.68)
Widowed/divorced	1.26	(0.84 - 1.88)	1.33	(1.04 - 1.71)	1.70	(1.31 - 2.20)	1.63	(1.41 - 1.88)

Table 2 continued

		Adjusted ORs AP		Adjusted ORs OQ		Adjusted ORs NQ		Adjusted ORs NP
Country of nationality								
Netherlands*	1.0		1.0		1.0		1.0	
Turkey	0.19	(0.03 - 1.41)	0.33	(0.13- 0.83)	0.21	(0.06 - 0.67)	1.73	(1.18 - 2.53)
Morocco	1.32	(0.62 - 2.83)	0.34	(0.14 - 0.79)	0.54	(0.26 - 1.11)	0.83	(0.54 - 1.27)
Other	1.29	(0.66 - 2.51)	1.09	(0.68 - 1.76)	1.46	(0.93 - 2.29)	3.26	(2.54 - 4.19)
Reminder								
By telephone*	1.0		1.0		1.0		1.0	
By mail	1.49	(1.22 - 1.82)	0.62	(0.55 - 0.70)	2.29	(1.99 - 2.64)	1.60	(1.47 - 1.73)
Other	2.58	(1.26 - 5.30)	4.97	(3.61 - 6.85)	5.19	(3.36 - 8.02)	8.11	(6.17 - 10.7)
Degree of urbanization								
Very high	2.26	(1.67 - 3.06)	1.31	(1.07 - 1.59)	2.76	(2.23 - 3.43)	2.21	(1.94- 2.52)
High	1.42	(1.02 - 1.98)	1.04	(0.85 - 1.27)	1.95	(1.54- 2.47)	1.17	(1.01 - 1.35)
Moderate	1.43	(1.07 - 1.91)	1.03	(0.87 - 1.22)	1.75	(1.42 - 2.16)	1.07	(0.95 - 1.21)
Low	1.01	(0.73 - 1.40)	0.99	(0.83 - 1.19)	1.12	(0.89 - 1.40)	1.02	(0.90 - 1.15)
No*	1.0		1.0		1.0		1.0	
Region								
Central	0.62	(0.44 - 0.89)	1.03	(0.85 - 1.25)	0.71	(0.57 - 0.90)	0.92	(0.80 - 1.06)
Southeast	1.08	(0.79 - 1.48)	1.01	(0.84 - 1.22)	0.63	(0.50 - 0.79)	0.85	(0.75 - 0.97)
Northwest	1.13	(0.81 - 1.57)	1.11	(0.91 - 1.34)	0.80	(0.63 - 1.00)	1.01	(0.88 - 1.15)
Southwest	0.89	(0.63 - 1.25)	1.13	(0.93 - 1.37)	0.80	(0.64 - 1.01)	0.89	(0.78 - 1.03)
Northeast*	1.0		1.0		1.0		1.0	

* Reference group; † Women aged 30-64 years versus men aged 30-64 years: AP adjusted OR 0.68 (95% CI 0.50 - 0.91); OQ adjusted OR 0.86 (95% CI 0.68 -1.08); NQ adjusted OR 0.64 (95% CI 0.51 - 0.81); NP adjusted OR 0.56 (95% CI 0.49 -0.63).

Table 3. Odds ratios (ORs) and 95 percent confidence intervals (95% CI) for characteristics comparing nonparticipants with questionnaire data with participants; Pienter Project, 1995-1996, The Netherlands

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
A: "Nonquestionnaire" variables					
Age group (years) by gender					
Male					
0-4	1,293	3.01	(2.57 - 3.54)	2.51	(2.00 - 3.16)
5-14	689	0.78	(0.61 - 0.99)	0.65	(0.49 - 0.86)
15-29	719	1.94	(1.60 - 2.37)	1.60	(1.26 - 2.05)
30-64*	1,892	1.0		1.0	
65-79	670	0.99	(0.78 - 1.23)	0.94	(0.74 - 1.18)
Female					
0-4	1,219	3.70	(3.13 - 4.36)	3.18	(2.52 - 4.00)
5-14	681	1.31	(1.04 - 1.64)	1.06	(0.81 - 1.41)
15-29	883	1.75	(1.44 - 2.12)	1.52	(1.20 - 1.92)
30-64*,†	2,110	1.0		1.0	
65-79	874	1.80	(1.48 - 2.19)	1.58	(1.29 - 1.94)
Marital status					
Married*	4,366	1.0		1.0	
Unmarried	5,809	2.23	(2.02 - 2.46)	1.37	(1.14 - 1.64)
Widowed/divorced	855	1.73	(1.45 - 2.06)	1.49	(1.24 - 1.80)
Country of nationality					
Netherlands*	10,697	1.0		1.0	
Turkey	70	0.40	(0.19 - 0.84)	0.29	(0.13 - 0.61)
Morocco	93	0.60	(0.34 - 1.04)	0.42	(0.24 - 0.75)
Other	170	1.26	(0.90 - 1.76)	1.14	(0.80 - 1.63)

Table 3 continued

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
Reminder					
By telephone*	5,738	1.0		1.0	
By mail	5,066	1.05	(0.96 - 1.15)	1.03	(0.94 - 1.14)
Other	226	5.55	(4.21 - 7.32)	4.82	(3.61 - 6.44)
Degree of urbanization					
Very high	1,377	1.66	(1.44 - 1.91)	1.67	(1.43 - 1.95)
High	1,384	1.21	(1.05 - 1.41)	1.30	(1.11 - 1.53)
Moderate	2,857	1.13	(1.00 - 1.27)	1.22	(1.06 - 1.40)
Low	2,275	1.00	(0.88 - 1.14)	1.03	(0.89 - 1.20)
No*	3,137	1.0		1.0	
Region					
Central	2,215	0.97	(0.84 - 1.11)	0.91	(0.78 - 1.07)
Southeast	2,253	0.89	(0.77 - 1.02)	0.82	(0.70 - 0.95)
Northwest	2,174	1.05	(0.92 - 1.21)	0.96	(0.82 - 1.12)
Southwest	2,181	1.13	(0.99 - 1.30)	0.98	(0.83 - 1.14)
Northeast*	2,210	1.0		1.0	
B: "Questionnaire" variables					
Opinion of importance of immunisation					
D, T, P, IPV, Hib, M, Me, R necessary*	7,443	1.0		1.0	
Seven of D, T, P, IPV, Hib, M, Me, R necessary	853	1.12	(0.96 - 1.31)	1.25	(1.06 - 1.49)
D, T, P, IPV, Hib, M, Me, R unnecessary	55	1.97	(1.15 - 3.37)	2.33	(1.33 - 4.10)
M, Me, R necessary, D, T, P, IPV, Hib other	267	1.41	(1.09 - 1.83)	1.72	(1.30 - 2.27)
D, T, P, IPV, Hib necessary, M, Me, R other	478	1.08	(0.88 - 1.32)	1.16	(0.92 - 1.44)
Other	1,934	1.34	(1.20 - 1.49)	1.59	(1.41 - 1.79)

Table 3 continued

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
Educational level**					
Low*	5,115	1.0			
Intermediate	3,308	1.26	(1.13 - 1.39)		
High	2,427	1.22	(1.09 - 1.36)		
Self-perception of health**					
(Very) good*	9,085	1.0			
Less than good	1,827	0.90	(0.80 - 1.02)		
Religion					
Orthodox Reformed	90	1.62	(1.06 - 2.47)		
Reformed Bond	145	0.97	(0.67 - 1.40)		
Other*	10,795	1.0			

* Reference group

† Women aged 30-64 years versus men aged 30-64 years: crude OR 0.78 (95% CI 0.67 - 0.94), adjusted OR 0.78 (95% CI 0.66 - 0.93)

** N < 11,030 due to missing values

The exclusion of absolute nonparticipants affects the association with nonparticipation for some "nonquestionnaire" variables considerably (Tables 1 versus 3). The odds ratios for 0-4 year-olds, Turks and Moroccans deviate farther from unity in the model restricted to groups with "questionnaire" variables (Table 3) than the odds ratios in the analysis with all (non)participant groups (Table 1). In contrast, the odds ratios for 5-14-year-olds, other nationality, and reminder by mail were closer to unity in the analysis restricted to (non)participants with questionnaire information.

The effect of considering additional participants as nonparticipants would only slightly affect the association with nonparticipation as most of the 95% confidence intervals include unity. For degree of urbanization and kind of reminder, the difference between participants and nonparticipants is slightly less when additional participants are included, while the difference increases slightly for nationality.

Table 4. Odds ratios (ORs) and 95 percent confidence intervals (95% CI) for opinion of immunisation categories comparing additional participants (AP; n=455), nonparticipants with a full questionnaire (OQ; n=1,618) and nonparticipants with a nonresponse questionnaire (NQ;1,053) with initial participants (IP; n=7,904) (reference group); Pienter Project, 1995-1996, The Netherlands

		Adjusted ORs* AP		Adjusted ORs* OQ		Adjusted ORs* NQ
Opinion of importance of immunisation						
D, T, P, IPV, Hib, M, Me, R necessary†	1.0		1.0		1.0	
Seven of D, T, P, IPV, Hib, M, Me, R necessary	0.75	(0.50 - 1.13)	1.42	(1.16 - 1.75)	1.04	(0.80 - 1.34)
D, T, P, IPV, Hib, M, Me, R unnecessary	0.60	(0.08 - 4.48)	2.01	(0.99 - 4.10)	2.65	(1.25 - 5.62)
M, Me, R necessary, D, T, P, IPV, Hib other	1.10	(0.60 - 2.01)	2.34	(1.68 - 3.26)	1.12	(0.72 - 1.73)
D, T, P, IPV, Hib necessary, M, Me, R other	0.73	(0.43 - 1.24)	1.25	(0.95 - 1.64)	0.98	(0.71 - 1.37)
Other	0.99	(0.76 -1.28)	1.81	(1.56 - 2.11)	1.32	(1.11 - 1.56)

* Adjusted for age group by gender, marital status, nationality, kind of reminder, degree of urbanization and region.

† Reference group

Discussion

The only way to eliminate bias from selection is to take a random sample and to achieve complete response, which is impossible in practice (15). The participation rate of 55% in our study was higher than expected, but differences found between participants and nonparticipants imply that our serum collection might not be representative of the general population (9,16).

When these differences are also associated with immune status of the disease in question they could lead to incorrect estimates of seroprevalence. Since the serum collection will be used for many seroprevalence studies - mainly directed to various vaccine-preventable diseases - this association should be studied separately for each seroprevalence study. Below we summarize for which characteristics differences were found between the (non)participation groups and whether associations with immune status are expected.

The likelihood of participation was lower for men than women for those aged 15-64 years. Some found that the participation rate was not related to gender, others reported that men were more difficult to recruit (3,5,10,17-20). The participation rate of the men were lowest among 15-29-year-olds, while the participation rate of the women was lowest among the oldest and youngest age group. Frequently, but not always, it is reported that nonparticipants were older than participants (4-7, 20-24). The high participation rate of 5-14-year-olds -an age group recently vaccinated- might be explained by the perceived importance of the topic of the study (25). The low participation rate of the younger age group is probably related to parents' fear of possible ill effects of blood sampling on their young children.
Age and gender are likely to be associated with immune status for vaccine-preventable diseases. Seroprevalence will depend on age as a result of age-specific differences in chance of exposure to the pathogen, time since exposure, chance of vaccination and time since last vaccination (1). For example those born before the introduction of mass vaccination in 1952 with DTP-IPV are more likely to be exposed to diphtheria and poliomyelitis but are less likely to have received vaccination. Gender differences in seroprevalence can be expected due for example vaccinations given in military service. In our recent study on tetanus antitoxin antibodies men with military service history were more likely to have tetanus antitoxin antibodies (26).

The special efforts to enhance the response rate of the Turks and Moroccans (the largest groups with non-Dutch nationality) seemed successful; in contrast to our pilot-study, Turks had a participation rate similar to the Dutch, and Moroccans had an even a higher participation rate than the Dutch (13). Relatively fewer individuals with another nationality participated.
Due to differences in force of infection and immunisation programmes in country of origin, immune status is likely to depend on nationality. For example the likelihood on protective tetanus antitoxin levels for Turkish and Moroccan individuals was lower than for Dutch individuals and individuals with other nationalities (26). Since non-Dutch individuals account for only a small part of the population the impact on the overall estimate is likely to be small.

Like others, we found that relatively fewer unmarried individuals than married individuals participated (3,4,6,27). The finding that a lower degree of urbanization was associated with more participation, is consistent with other reports (18,24). Active refusers accounted for the largest part of the group described by "other reminder", which caused the high odds ratio for nonparticipation. Consistent with other studies individuals who could be reached and reminded by telephone participated more frequently than those who were reminded by mail (11, 16, 28).
Although the associations for these three characteristics with immune status are unclear, we expect them to be small. This has recently been supported by the absence of associations with tetanus immunity (26).

The polytomous logistic regression analysis showed that since additional participants resembled nonparticipants more for degree of urbanization and kind of reminder, the nonresponse bias due to these characteristics is slightly less than it would have been if these subjects had not participated. However, with respect to nationality, additional participants resembled initial participants more than nonparticipants, so that the reverse is true for this characteristic. Although including additional participants probably affected the nonresponse bias in our study very little, our results show that the assumption that additional (late) participants resemble nonparticipants more than initial participants as they both participated not in the first stage might be incorrect (9,11,19,29,30).

Some variables affected each form of nonparticipation similarly, while other variables influenced each form of nonparticipation differently. Identifying nonresponse bias in our study on the basis of completed questionnaires would overestimate the nonresponse bias for 0-4-year-olds and for Turks and Moroccans, and underestimate it for individuals of another non-Dutch nationality and for those reminded by mail.

This implies that our results from the analysis restricted to those with questionnaire information are also difficult to interpret. In this analysis, opinions about the importance of immunisation turned out to be an independent predictor for nonparticipation for those with questionnaire information. Individuals who considered none of the immunisations necessary, or M,Me, and R necessary and D,T,P,IPV, and Hib otherwise, were most likely to be nonparticipant (with questionnaire). In the univariate analysis, the likelihood of being a nonparticipant who filled in a questionnaire was higher for individuals with an intermediate or high educational level and who were Orthodox Reformed, and slightly lower for individuals who considered their health less than good. These findings are inconsistent with those in other studies for educational level and self-perception of health, while no nonresponse studies on the effect of opinion of importance of immunisation and religion are known (3-6,10,20-23).
Vaccination history and as a result of refusing vaccination orthodox reformed religion is expected to be associated with immune status to vaccine preventable diseases. Since the vaccine coverage in the Netherlands is high (97% for DTP-IPV, Hib; 94% for M, Me and R) and the group of orthodox reformed individuals is small (about 300,000) the impact on the seroprevalence estimates are probably limited. Again these expectations were supported by the seroprevalence study on tetanus (26).
In this population-based study addressing vaccine-preventable diseases differences were found between participants and nonparticipants as they were in other studies.
Correction for differential participation can be made by taking the distribution of the concerning characteristic in the Dutch population (if known, i.e. as for age) into account in the seroprevalence estimates. Furthermore, when information for all participants and nonparticipants

is known the effect on the seroprevalence can be estimated by weighting the seroprevalence estimate by differential response rate. However, the results of our polytomous logistic regression analysis show that when information is available only for a subgroup of nonparticipants it might lead to biased insight into nonresponse selection. If such a characteristic is associated with immune status of the disease in question, one can not be certain on the exact impact on the overall seroprevalence estimate. Furthermore, our study shows that investigators have to be aware that including additional participants might not always reduce nonresponse bias.

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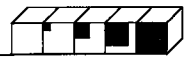

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Part 2

Immunosurveillance for diphtheria, tetanus and poliomyelitis

Chapter 4

Diphtheria antitoxin levels in the Netherlands: a population-based study

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Abstract

In a population-based study in the Netherlands, diphtheria antitoxin antibodies were measured with a toxin-binding inhibition assay in 9,134 sera from the general population and of religious communities refusing vaccination.

The Dutch immunisation programme appears to induce long-term protection against diphtheria. However, a substantial number of adults born before the programme was introduced had no protective diphtheria antibody levels. Although herd immunity seems adequate, long-term population protection cannot be assured. As more than 60% of orthodox reformed persons have diphtheria antibody levels lower than 0.01 IU/ml, introduction of diphtheria into religious communities refusing vaccination may constitute a danger of spread of the bacterium.

Introduction

The recent diphtheria epidemics in Eastern Europe are a warning that diphtheria can make a comeback in susceptible populations (1). The World Health Organization recommends the implementation of strategies for non-epidemic countries, including the assessment of the population diphtheria immunity status, to prevent any indigenous cases in the European Region by the year 2000 (2).

In the Netherlands the last diphtheria epidemic occurred during World War II (220,000 cases were reported in 1940 to 1946). Diphtheria vaccination was introduced in 1952 for persons born after 1945. Under the current schedule, children are vaccinated at ages 3, 4, 5, and 11 months with diphtheria, tetanus, pertussis and inactivated polio vaccine (DTP-IPV) and at ages 4 and 9 years with DT-IPV. For the past decades, the vaccine coverage for at least three vaccinations at the age of 12 months has been 97%. Rare exposure to *C. diphtheriae* may have led to lack of boosting opportunities (1). As in other developed countries, lack of immunity in older persons is a reason for concern (3,4). Furthermore, in the Netherlands, the immune status of socio-geographically clustered members of religious communities who refuse vaccination may be even more unfavourable. Inadequate diphtheria herd immunity in these groups could lead to outbreaks similar to the poliomyelitis outbreaks in the Netherlands (5). A large population-based serum bank allowed us to assess the diphtheria immunity in the Dutch population and in persons refusing vaccination (6).

The study

In October 1995 through December 1996, a population-based serum bank with specimen from 9,948 individuals was established (6). Our objective was to select 40 municipalities with sampling probabilities proportional to population size. In each of five regions, eight municipalities were included. For each of these 40 municipalities, an age-stratified sample of 380 individuals was drawn from the population register (7). Participants were requested to have a blood sample drawn, complete a questionnaire, and provide immunisation and military service records. Participants were also selected from eight additional municipalities with low vaccine coverage to assess the immunity of members of religous communities that refuse vaccination. The nationwide sample had 8,357 (55%) and the low vaccine coverage sample had 1,589 (52.5%). Sufficient serum was available for testing 7,715 of the nationwide participants and for 1,419 of the participants in the sample with low vaccine coverage.

Methods

Sera were stored at minus 86 °C. The level of diphtheria antitoxin antibodies was measured with a toxin-binding inhibition assay as described earlier (8). In brief, twofold serum dilution series were incubated with a fixed amount of toxin and the nonneutralised toxin was measured in an enzyme-linked immunosorbent assay (ELISA) with equine antitoxin purified from hyperimmune serum as coat and peroxidase-labelled horse antidiphtheria IgG as conjugate. International units were calculated according to the WHO reference standard serum (10 IU/ml) by the four parameter fit method in Kineticalc (KC4, Biolyse) with a Bio-Tek plate reader (EL312d). The minimum level of detection was 0.01 IU/ml and samples below this level were set to 0.005 IU/ml for calculating geometric mean titres. The good correlation with the Vero neutralisation assay was confirmed again recently ($r \geq 0.95$) (9).
Antitoxin antibody levels were classified according to international standards < 0.01 IU/ml (no protection), 0.01 IU/ml to 0.1 IU/ml (basic protection) and > 0.1 IU/ml (full protection) (10).

Analysis

Frequencies and geometric mean titres in each municipality were weighted by the proportion of the age group in the population. To produce national estimates, the weighted frequencies and geometric mean titres were averaged over the 40 municipalities (7). For the low vaccine coverage sample, they were averaged weighting by the population size of the municipality.
Data on age, sex, marital status, country of nationality, degree of urbanization, region and contact information for all participants and nonparticipants was available. The effect of differential probabilities of response for these variables on both sample estimates was less than one standard error and was therefore disregarded.
Linear regression analysis was used to study the persistence of diphtheria antitoxin antibodies after full immunisation in the national immunisation programme. The association between diphtheria antibody titre (^{2}log) and age in ^{2}log years was studied in this analysis for persons who received th sixth documented vaccination at 8 to 9 years of age, without self-reported or documented revaccination or military service history.

Age-specific dipththeria antitoxin immunity levels

In the nationwide sample 58.1%, 30.0% and 11.9% of persons ≤ 79 years of age had full, basic, or no diphtheria protection, respectively (Table 1). Women had lower levels of full protection and geometric mean titres. A greater percentage of persons from the municipalities with low vaccine coverage and of members of religous communities in this low vaccine coverage sample had no protection (Table 2). When members of religious communities were excluded from this low

vaccine coverage sample, i.e. for not orthodox reformed persons, the percentages of full, basic, and no protection were 57.3%, 25.2%, and 17.5%, respectively (Table 2).

For the ages of 1, 4 and 8 to 9 years the geometric mean titre and percentages of persons with full protection increased (Table 3). The percentage with full protection decreased after the age of 10 to 14 years, but increased for the 35- to 44-year age group (Figure 1).

After the age of 40 to 44 years, the percentage with full protection and the geometric mean titre decreased. Although the geometric mean titres differed statistically significantly by gender only after the age of 30 years, they were slightly lower for females from 5 to 9 years old and older (Table 3).

Both for orthodox reformed individuals less than 50 years and for those at least 50 years of age, the proportion with no protection was higher than for persons in the nationwide sample (Table 4). Men and women aged 20 to 49 years without a military service history had similar proportions of full, basic and no protection, while the proportion with full protection was higher for men with a military service history (Table 5).

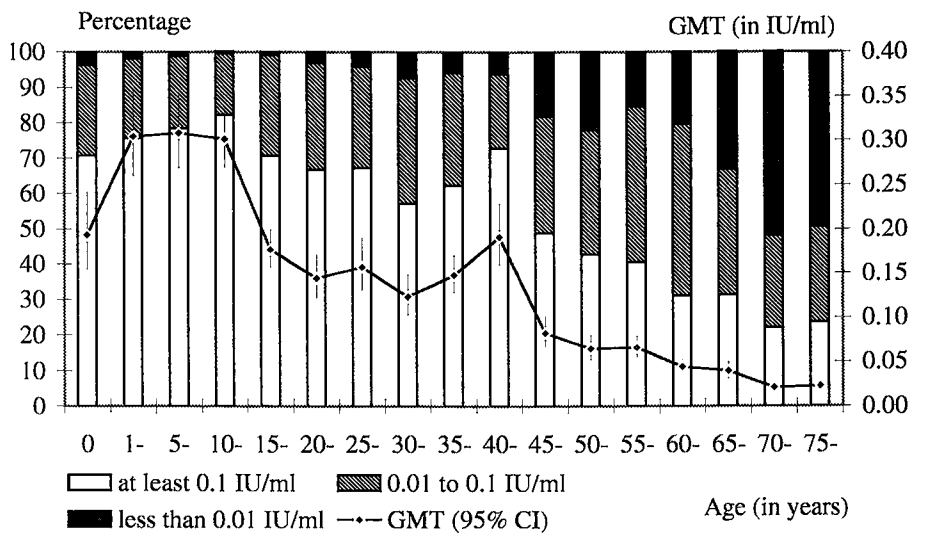

Figure 1. Age-specific diphtheria immunity in the nationwide sample (n = 7715), The Netherlands

Table 1. Diphtheria immunity in a nationwide sample of persons ≤ 79 years of age, The Netherlands

	Number	<0.01 IU/ml	(95% CI)	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	GMT	(95% CI)
Nationwide sample									
Overall	7715	11.9	(10.7 - 13.1)	30.0	(28.3 - 31.7)	58.1	(56.2 - 59.9)	0.12	(0.11 - 0.13)
Men	3644	9.3	(8.0 - 10.6)	28.1	(25.9 -30.2)	62.6	(60.1 - 65.2)	0.14	(0.13 - 0.16)
Women	4071	14.4	(12.6 - 16.2)	31.6	(29.7 - 33.6)	54.0	(51.9 - 56.0)	0.10	(0.09 - 0.11)

Table 2. Diphtheria immunity in the sample of persons ≤ 79 years of age with low vaccine coverage, The Netherlands

	Number	<0.01 IU/ml	(95% CI)	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	GMT	(95% CI)
Orthodox Reformed									
Overall	233	60.9	(42.9 - 78.9)	12.4	(6.0 - 18.7)	26.7	(13.8 - 39.7)	0.02	(0.01 - 0.04)
Men	116	69.7	(54.2 - 85.2)	10.4	(4.2 - 16.6)	19.9	(8.2 - 31.5)	0.01	(0.01 - 0.03)
Women	117	52.3	(27.7 -76.8)	15.0	(6.9 - 23.2)	32.7	(15.3 - 50.1)	0.03	(0.01 -0.06)
Not Orthodox Reformed									
Overall	1259	17.5	(15.5 - 19.4).	25.2	(21.3 - 29.2)	57.3	(52.2 - 62.4)	0.10	(0.09 - 0.12)
Men	590	11.5	(8.2 - 14.8)	25.5	(20.5 - 30.6)	63.0	(55.4 - 70.5)	0.14	(0.13 - 0.16)
Women	669	21.7	(18.0 - 25.4)	25.0	(21.2 - 28.9)	53.3	(48.6 - 58.0)	0.08	(0.07 - 0.10)
Total low vaccine coverage sample									
Overall	1492	24.3	(20.5 - 28.0)	23.6	(20.1 - 27.1)	52.1	(48.2 - 56.0)	0.08	(0.07 -0.09)
Men	706	20.6	(16.6 - 24.7)	23.4	(18.9 - 27.8)	56.0	(50.3 - 61.7)	0.10	(0.08 - 0.12)
Women	786	26.7	(21.1 - 32.4)	23.3	(19.9 - 26.7)	50.0	(45.3 - 54.7)	0.07	(0.06 - 0.09)

Table 3. Age-specific prevalence of diphtheria immunity and geometric mean titres ≤ 14 years of age and for men and women less than 79 years op age in the nationwide sample, The Netherlands

Age group (years)	Number	<0.01 IU/ml	(95% CI)	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	GMT	(95% CI)
0	187	3.7	(1.1 - 6.4)	25.5	(18.6 - 32.4)	70.7	(63.4 - 78.1)	0.19	(0.15 - 0.24)
1	185	1.2	(0.0 - 3.0)	9.9	(5.4 - 14.3)	88.9	(84.3 - 93.5)	0.57	(0.45- 0.72)
2	156	1.9	(0.1 - 3.7)	34.9	(24.2 - 45.5)	63.3	(52.9 - 73.6)	0.15	(0.12- 0.19)
3	215	1.7	(0.1 - 3.3)	35.5	(27.7 - 43.3)	62.8	(54.7 - 70.9)	0.16	(0.12- 0.22)
4	153	0.6	(0.0 - 1.7)	8.7	(3.7 - 13.7)	90.7	(85.7 - 95.7)	0.81	(0.60- 1.10)
5	102	1.4	(0.0 - 3.3)	6.3	(1.9 - 10.7)	92.3	(87.7 - 97.0)	0.43	(0.35- 0.52)
6	121	0.9	(0.0 - 2.6)	20.0	(11.7 - 28.3)	79.1	(70.9 - 87.4)	0.26	(0.21- 0.34)
7	101	2.3	(0.0 - 5.4)	31.8	(20.3 - 43.3)	65.9	(54.7 - 77.2)	0.16	(0.13- 0.20)
8	127	0.7	(0.0 -2.0)	23.6	(14.9 - 32.3)	75.8	(66.9 - 84.6)	0.28	(0.20- 0.38)
9	97	0.0	(0.0 - 0.0)	11.8	(3.2 - 20.4)	88.2	(79.6 - 96.8)	0.71	(0.51- 0.99)
10	113	0.7	(0.0 - 2.1)	6.7	(1.9 - 11.5)	92.6	(87.7 - 97.5)	0.54	(0.42- 0.69)
11	111	0.7	(0.0 - 1.9)	8.3	(3.5 - 13.2)	91.0	(85.8 - 96.2)	0.36	(0.30- 0.44)
12	122	0.6	(0.0 - 1.9)	17.0	(9.2 - 24.8)	82.4	(74.2 - 90.5)	0.28	(0.22- 0.35)
13	126	0.0	(0.0 - 0.0)	27.3	(18.0 - 36.7)	72.7	(63.3 - 82.0)	0.23	(0.18- 0.29)
14	102	0.4	(0.0 - 1.1)	26.7	(14.0 - 39.3)	72.9	(60.3 - 85.6)	0.22	(0.15- 0.31)
Men									
0	104	3.5	(0.04 - 7.0)	25.2	(15.8 - 34.6)	71.3	(61.4 - 81.2)	0.18	(0.13 - 0.24)
1-4	376	1.8	(0.5 - 3.1)	22.9	(17.0 - 28.8)	75.3	(69.1 - 81.5)	0.31	(0.25 - 0.38)
5-9	296	0.6	(0.0 - 1.4)	16.9	(12.4 - 21.4)	82.5	(77.9 - 87.1)	0.34	(0.28 - 0.40)
10-14	280	0.6	(0.0 - 1.7)	16.5	(11.7 - 21.3)	83.0	(78.0 - 88.0)	0.32	(0.27 - 0.38)
15-19	209	1.0	(0.0 - 2.4)	25.8	(19.7 - 31.9)	73.1	(67.1 - 79.1)	0.19	(0.16 - 0.23)
20-24	139	4.0	(0.4 - 7.6)	25.5	(17.8 - 33.2)	70.5	(62.0 - 79.0)	0.15	(0.12 - 0.19)
25-29	150	2.9	(0.0 - 6.2)	30.6	(21.7 - 39.5)	66.5	(56.9 - 76.1)	0.18	(0.14 - 0.24)
30-34	188	7.0	(1.6 - 12.4)	31.5	(23.0 - 40.0)	61.5	(52.1 - 70.9)	0.14	(0.10 - 0.19)

Table 3 Continued.

35-39	220	5.1	(1.3 - 8.9)	27.4	(20.7 - 34.1)	67.5	(60.8 - 74.2)	0.18	(0.14 - 0.23)
40-44	230	3.8	(1.1 - 6.5)	16.3	(11.0 - 21.6)	79.9	(73.9 - 85.9)	0.25	(0.20 - 0.31)
45-49	208	13.7	(7.8 - 19.6)	26.6	(21.4 - 31.8)	59.7	(51.8 - 67.6)	0.13	(0.09 - 0.18)
50-54	228	15.0	(10.4 - 19.6)	31.1	(24.7 - 37.5)	53.9	(46.5 - 61.3)	0.10	(0.08 - 0.13)
55-59	251	8.8	(4.0 - 13.6)	38.8	(32.1 - 45.5)	52.4	(46.0 - 58.8)	0.10	(0.08 - 0.12)
60-64	216	10.3	(6.2 - 14.4)	52.3	(43.8 - 60.8)	37.4	(29.9 - 44.9)	0.07	(0.06 - 0.09)
65-69	200	26.5	(19.6 - 33.4)	39.4	(30.4 -48.4)	34.1	(26.2 - 42.0)	0.05	(0.04 - 0.06)
70-74	193	46.2	(36.9 - 55.5)	26.6	(19.2 - 34.0)	27.1	(19.2 - 35.2)	0.03	(0.02 - 0.04)
75-79	156	45.0	(35.8 - 54.2)	26.2	(17.8 - 34.6)	28.8	(20.8 - 36.8)	0.03	(0.02 - 0.04)
Women									
0	83	4.2	(0.0 - 8.9)	26.6	(13.7 - 39.5)	69.3	(56.7 - 81.9)	0.19	(0.13 - 0.27)
1-4	333	2.2	(0.5 - 3.9)	22.2	(16.7 - 27.7)	75.6	(70.0 - 81.2)	0.30	(0.25 - 0.37)
5-9	252	1.8	(0.0 - 3.7)	23.8	(16.2 - 31.4)	74.5	(68.2 - 80.8)	0.29	(0.24 - 0.36)
10-14	294	0.3	(0.0 - 0.8)	18.6	(12.9 - 24.3)	81.1	(75.4 - 86.8)	0.29	(0.24 - 0.33)
15-19	243	0.7	(0.0 - 1.7)	28.2	(21.9 - 34.5)	71.1	(64.8 -77.4)	0.17	(0.15 - 0.20)
20-24	199	2.8	(0.4 - 5.2)	31.3	(23.6 - 39.0)	65.9	(58.3 - 73.5)	0.14	(0.12 - 0.18)
25-29	226	5.0	(1.5 - 8.5)	27.1	(20.3 - 33.9)	67.9	(60.5 - 75.3)	0.14	(0.11 - 0.18)
30-34	244	10.0	(5.7 - 14.3)	36.9	(30.5 - 43.3)	53.0	(47.3 - 58.7)	0.11	(0.09 - 0.13)
35-39	282	5.6	(2.5 - 8.7)	37.0	(29.6 - 44.4)	57.4	(50.6 - 64.2)	0.13	(0.11 - 0.15)
40-44	247	8.7	(4.8 - 12.6)	24.4	(18.5 - 30.3)	66.9	(60.9 - 72.9)	0.16	(0.12 - 0.20)
45-49	261	22.0	(15.8 - 28.2)	37.6	(30.6 - 44.6)	40.3	(32.3 - 48.3)	0.06	(0.04 - 0.08)
50-54	264	28.2	(20.2 - 36.2)	41.7	(34.5 - 48.9)	30.1	(23.0 - 37.2)	0.04	(0.03 - 0.05)
55-59	250	22.3	(16.3 - 28.3)	49.7	(43.6 - 55.8)	28.0	(22.3 - 33.7)	0.04	(0.03 - 0.05)
60-64	237	29.2	(22.9 - 35.5)	45.9	(39.7 - 52.1)	24.8	(19.0 - 30.6)	0.03	(0.02 - 0.04)
65-69	263	38.0	(30.6 - 45.4)	34.3	(29.0 - 39.6)	27.7	(21.3 - 34.1)	0.03	(0.02 - 0.05)
70-74	218	58.2	(50.8 - 65.6)	25.0	(18.9 - 31.1)	16.8	(11.0 - 22.6)	0.02	(0.01 - 0.02)
75-79	175	52.7	(43.8 - 61.6)	26.6	(19.4 - 33.8)	20.7	(12.7 - 28.7)	0.02	(0.01 - 0.03)

Table 4. Diphtheria immunity in the nationwide sample and in orthodox reformed individuals in municipalities with low vaccine coverage, The Netherlands

	Number	<0.01 IU/ml	(95% CI)	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	GMT	(95% CI)
Nationwide sample									
0 - 49 years	5064	5.3	(4.4 - 6.2)	27.3	(25.3 - 29.4)	67.4	(65.2 - 69.5)	0.17	(0.16 - 0.19)
50 -79 years	2651	29.2	(26.4 - 31.9)	37.4	(35.3 - 39.5)	33.4	(31.3 - 35.6)	0.04	(0.04 - 0.05)
Orthodox reformed from low vaccine coverage sample									
0 - 49 years	170	60.8	(40.2 - 81.4)	7.4	(2.3 - 12.6)	31.8	(13.8 - 49.7)	0.02	(0.01 - 0.05)
50 -79 years	63	59.3	(30.1 - 88.6)	34.6	(9.5 - 59.6)	6.1	(0.0 - 18.2)	0.01	(0.005 - 0.025)

Table 5. Diphtheria immunity in the nationwide among persons 20 to 49 years, according to sex and military service, The Netherlands

	Number	<0.01 IU/ml	(95% CI)	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	GMT	(95% CI)
Military service* (20-49)									
Men with military service history	425	2.9	(1.2 - 4.6)	14.9	(11.1 - 18.7)	82.2	(78.0 - 86.5)	0.30	(0.25 - 0.36)
Men without military service history	710	7.8	(5.2 - 10.3)	33.1	(28.0 - 38.1)	59.2	(54.0 -64.4)	0.12	(0.10 - 0.14)
Women without military service history	1456	9.1	(7.0 - 11.2)	32.5	(29.0 - 36.0)	58.4	(54.5 - 62.3)	0.12	(0.10 - 0.13)

* Subgroup of the nationwide sample

Persistence of diphtheria antitoxin levels

The geometric mean titre decreased with age (or time since last vaccination) for persons who had received their last and sixth vaccination at 8 to 9 years of age (n=961) from 0.30 IU/ml for 10 to 14 years to 0.09 IU/ml for 30 to 34 years (Table 6, Figure 2). According to linear regression analysis, the decrease corresponds to a decrease of -1.27 ^{2}log IU/ml with each ^{2}log increase in years in age. The percentage with full protection decreased from 82.5% to 41.7%, and the percentage with no protection increased from 0% to 4.3% for these 10 to 14 year olds and 30 to 34 year olds, respectively (Table 6).

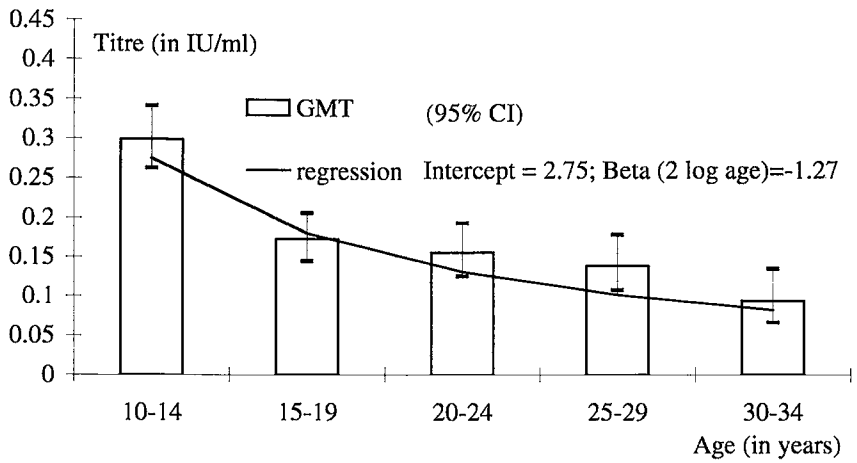

Figure 2. Diphtheria antitoxin titre (Geometric mean titre (GMT) ± 2 standard error) by age group and linear regression of diphtheria antitoxin antibody titre (in ^{2}log) with age (in ^{2}log years) for individuals with the sixth and last documented vaccination at the age of 8 or 9 years (n = 961) in the nationwide sample, The Netherlands

The geometric mean titre for persons 20 to 34 years with documented revaccination (n=37) was 0.29 IU/ml. Percentages of full (81.0%), basic (19.0%), and no (0.0%) protection were similar to recently vaccinated 10 to 14 year olds, without further documented or reported revaccination (Table 6).

Table 6. Diphtheria immunity for persons in the nationwide sample who were completely vaccinated in the national immunisation programme and with the sixth documented vaccination at 8 or 9 years of age, The Netherlands

Age group	Number	<0.01 IU/ml	(95% CI)	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	GMT	(95% CI)
No evidence for revaccination									
10-14	392	0.0	--	17.5	(12.8 - 22.1)	82.5	(77.9 - 87.1)	0.30	(0.26-0.34)
15-19	282	0.4	(0.0 - 1.3)	30.1	(23.7 - 36.5)	69.4	(63.2 - 75.7)	0.17	(0.14-0.21)
20-24	155	1.2	(0.0 - 3.0)	29.4	(20.7 -38.2)	69.4	(60.7 - 78.1)	0.16	(0.12-0.19)
25-29	80	1.6	(0.0 - 3.7)	28.3	(17.2 - 39.4)	70.2	(58.7 - 81.6)	0.14	(0.11-0.18)
30-34	52	4.3	(0.0 - 9.2)	54.0	(39.3 - 68.8)	41.7	(27.3 - 56.1)	0.09	(0.07-0.13)
Evidence for revaccination									
20-34	37	0.0	--	19.0	(3.2 - 34.9)	81.0	(65.1 - 96.8)	0.29	(0.18 - 0.46)

Conclusions

Our population-based study showed that 58% of the Dutch population had full, 30% basic, and 12%, no protection against diphtheria. These estimates and the geometric mean titre (0.12 IU/ml) are in between findings for other European countries (4,11-20). The Dutch immunisation programme appeared to induce long-term protection. However, approximately one third of adults aged 50 to 79 years, who born before the introduction of the immunisation programme and approximately two-thirds of orthodox reformed persons had no protective diphtheria antibodies.

The toxin inhibition test to measure diphtheria antitoxin concentrations shows good correlation with the in vitro neutralisation test in Vero cells, but is faster, simpler, and combines the measurement of diphtheria and tetanus antitoxin antibodies (8,9).
Although the participation rates in the nationwide sample and low vaccine coverage sample amounted to 55% and 52.5% respectively, our population-based estimates of diphtheria immunity were considered representative, because they seem to be hardly affected by non-participation.

Our participants included a large percentage of persons with diphtheria protection who were born after the vaccination was introduced in 1952 and after the virtual disappearance of diphtheria in 1960. High levels of immunity in this group reflect the success of the national vaccination programme.
For persons born before the introduction of vaccination, diphtheria immunity is largely derived from natural infection. However, immunity levels in those older than 49 years in the general population are higher than those of orthodox reformed persons suggesting that immunity was partly induced by vaccinations (e.g., for military service, travel).
The sharp increase in the percentage of persons older than 44 years with no protective diphtheria antitoxin levels is consistent with findings of other studies (4,11,12,14-19). It supports the phenomenon of waning immunity after natural infection without boosting.

In our study, higher immunity levels among men are associated with military service, as previously reported (15,19). However, other researchers have found similar immunity levels for men and women, while others have reported lower immunity for men (11,16,21). Furthermore, lower immunity for women that could not be ascribed to vaccinations during military service has also been reported (4,20). Women might maintain immunity after vaccination for a shorter time than men (14). The slightly lower geometric mean titres for girls aged 5 to 19 years in our study are consistent with the latter possibility.
As more than 60% of orthodox reformed persons have no protection against diphtheria, introduction of diphtheria into this sociogeographically clustered group may constitute a substantial danger of spreading the bacterium.

Since the Netherlands does not have a mandatory vaccination policy, protection of persons who refuse vaccination is problematic. For poliomyelitis the solution seems to be eradication of the causative agent (5). For diphtheria such a goal has not yet been formulated by the World Health Organization. However, even though systematic assessment has not been performed, no signs of persistent circulation of *C. diphtheriae* exists in the Netherlands.

When our data are interpreted longitudinally, the decrease in diphtheria antibody level with age for individuals completely vaccinated in the national immunisation programme, corresponds with a continuous decline of vaccine-induced antibodies (13,22). However, relatively few 30 to 34-year-old persons (4.3 percent) who received their last vaccination approximately 25 years ago had a diphtheria antitoxin level of less than 0.01 IU/ml. This compares favourable with observations in other countries (13,21-23). Our immunisation programme, in which children are vaccinated at 3, 4, 5 and 11 months with 15 Lf diphtheria toxoid, and at the ages of 4 and 9 years with 2.5 Lf appears to induce long-term protection against diphtheria.

In the Netherlands, booster vaccinations are only advised for individuals at increased risk for exposure (e.g. travellers to endemic countries, and those who work with injection drug users, alcoholic patients).
The need for routine boosters to guarantee population protection depends mainly on the proportion necessary to confer diphtheria herd immunity. This proportion is estimated at 70% to 80%, but no antitoxin level has been precisely defined for complete protection (10,13, 24-26). The Dutch immunity level exceeds this threshold (a minimum level of 0.01 IU/ml [88%]), but is below a minimum level of 0.1 IU/ml (58%).
The absence of cases in the Netherlands associated with the diphtheria epidemic in Eastern Europe suggests that herd immunity is sufficient. This herd immunity might be the result from sufficient protective levels of antitoxin and/or of immunologic memory. Our results, like those of others, indicate good immunologic memory after revaccination for persons who had been previously vaccinated (17,27). However, the (memory) response of adults after initial vaccination is unknown. Furthermore, unknown protective mechanisms might be involved. Only sporadic cases and no outbreaks have occurred, in other European countries where gaps have been found in the diphtheria antitoxin levels of adults. The only recent epidemic in Western Europe, which occurred before the epidemic in Eastern Europe, was one among alcoholics (23). Perhaps unfavourable social conditions, such as appear to have contributed to the epidemics in Eastern Europe, play a crucial role in spread of diphtheria (1).

In conclusion, a substantial percentage of adults born before the introduction of the immunisation programme have low diphtheria antitoxin levels. Although herd immunity seems sufficient, long-term population protection cannot be assured. Possibly vaccination might fill the gaps of

diphtheria antitoxin antibodies. Diphtheria vaccination could be efficiently combined with other vaccines (e.g., tetanus, influenza) as part of an adult immunisation programme.

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Chapter 5

A population-based study on tetanus antitoxin levels in the Netherlands

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Abstract

We assessed the tetanus immunity of the general Dutch population and of religious groups refusing vaccination by means of population-based study to evaluate the effect of tetanus vaccination. More than 95% of those born after the introduction of routine vaccination had tetanus antitoxin levels above the minimum protective level. After the sixth vaccination, a fall in tetanus antitoxin levels occurred. Nevertheless, immunisation in accordance with the routine programme most likely induces protection for much longer than two decades. Not only many members of religious groups who refuse vaccination, but also many adults born before the introduction of vaccination lack tetanus immunity. These cohorts might benefit most from (re)vaccination.

Introduction

Vaccination with tetanus toxoid, one of the most effective prophylactic procedures, has greatly reduced the incidence of tetanus (1). In the past decade, two to three cases has been reported annually in the Netherlands; these occurred mainly among individuals born before the introduction of childhood vaccination (2). Mass vaccination was introduced in the Netherlands in 1952 for people born in 1945 and thereafter. Children are vaccinated at 3, 4, 5 and 11 months of age with a diphtheria, tetanus, pertussis and inactivated polio vaccine (DTP-IPV) and at 4 and 9 years of age with DT-IPV.

Tetanus is not directly communicable between hosts; therefore, vaccination cannot confer herd immunity (3). Effective control requires immunising every individual by vaccination. Although some have reported natural immunity, it is believed that immunity to tetanus toxin is induced only by vaccination (1).

The prevalence of specific antibodies can be used as a parameter to evaluate the effects of vaccination. It offers us the opportunity of finding clusters of individuals susceptible to tetanus. This is particularly important in the Netherlands because of its geographically clustered religious groups who refuse vaccination. Furthermore, by studying the persistence of tetanus antibodies after vaccination through the national programme, possible vaccination requirements for providing continuous immunity to tetanus can be evaluated.

Our large-scale, population-based, serum bank gave us an opportunity of studying the tetanus immunity of the Dutch population and of individuals refusing vaccination (4).

Material and methods

Study population

Eight municipalities with probabilities proportional to their population sizes were sampled within each of five geographical Dutch regions with similar population sizes. An age-stratified sample (classes 0, 1-4, 5-9 to 75-79 years) of 380 individuals was randomly selected from each municipality. These individuals were requested to give a blood sample, fill out a questionnaire and bring their vaccination certificates from the national immunisation programme and military service, as well as proof of travel vaccinations. Similarly, individuals were selected from eight municipalities with low vaccine coverage to assess the immunity in geographically clustered orthodox reformed groups that refuse vaccination. The data collection was carried out in October 1995 to December 1996. The participation rates were 55% and 52.5% in the nationwide sample and the low vaccine coverage sample, respectively. The study design and details on the data collection have been published elsewhere (4).

Antibody assay

Sera were stored at minus 86 °C. The level of tetanus antitoxin antibodies was measured with a toxin binding inhibition (ToBI) assay. The results were standardised with the four-parameter-fit method, and the national reference serum calibrated on the WHO international reference serum was used. The ToBI assay shows a good correlation with the in vivo neutralisation test, even for sera with low titres up to a concentration of 0.01 IU per ml (5-8). Sufficient serum was available for 7715 of the 8359 of the participants of the nationwide sample and for 1492 of the 1589 participants of the low vaccine coverage sample.
According to internal standards, an antitoxin level of 0.01 IU/ml was considered the minimum protective level (1). A titre of less than 0.01 IU/ml was set to 0.005 IU/ml for calculating geometric mean titres (GMTs).

Definition of variables

Two religious groups were distinguished: "orthodox reformed" (opposed to vaccination), and "other" (9). Documented revaccination was defined as any documented vaccination given in addition to vaccinations documented on the certificate of the national immunisation programme. For those with documented revaccination the median number of revaccinations was two and ranged from one to six.
The questionnaire asked participants of all age groups whether they had received a tetanus vaccination after injury, and those aged at least 17 years whether they had received a vaccination for diphtheria, tetanus and poliomyelitis (DT-IPV) for military service, travel or profession.

The educational levels of those aged 17 years or more and of one of the parents for those aged less than 17 years, were classified as "low" (primary school, lower vocational or lower general secondary education), "intermediate" (intermediate vocational or intermediate general secondary and higher general secondary education) and "high" (higher vocational secondary education and university education). The following categories for degree of urbanisation were made: "very high" (>2,500 addresses/km^2), "high" (1,500-2,500), "moderate" (1,000-1,500), "low" (500-1,000), and "none" (< 500). The geographical regions were based on the Dutch provinces: "central" (Utrecht and Gelderland), "southeast" (Brabant and Limburg), "northwest" (North Holland and Flevoland), "southwest" (Zeeland and South Holland) and "northeast" (Groningen, Drente, Overijssel and Friesland).

In logistic regression analysis (see the statistical analysis), evidence of tetanus vaccination was coded as 'yes' if the participant: i.) had documented tetanus vaccination, ii.) reported a military

service history; iii.) reported participation in the national immunisation programme or iv.) reported vaccination against DT-IPV or against tetanus after an injury.

Statistical analysis

Frequencies and GMTs within each municipality were weighted by the proportion of the age group in the population. To produce national estimates, the weighted frequencies and GMTs were averaged over the 40 municipalities (10). For the low vaccine coverage sample, they were averaged weighting by the population size of the municipality. The effect of differential probabilities of response on both sample estimates was less than one standard error and was therefore ignored.

Linear regression analysis was used to study the persistence of tetanus antitoxin antibodies after complete participation in the national immunisation programme. The association between the tetanus antibody titre (^{2}log) and the inverse of age was studied in this analysis for individuals with the sixth documented vaccination at 8 to 9 years of age, and without self-reported or documented revaccination or military service history. This analysis was restricted to those aged 10 to 34 years of age, as for older age groups the number of individuals with documentation of six vaccinations was too small.

Logistic regression analysis was conducted to determine whether any of the following variables were independent predictors of a tetanus antitoxin level of less than 0.01 IU/ml in the nationwide sample after adjustment for age group by sex: marital status, educational level, region, degree of urbanisation, evidence of tetanus vaccination, orthodox reformed religion, country of birth and self-reported travel to Eastern Europe, Turkey or Greece, Asia, Central America, South America, North Africa, and Central Africa or South Africa. Variables remained in the multivariate model if either the likelihood ratio test was significant ($p < 0.05$) or the estimates of the beta coefficients for other variables in the model changed by at least 10%.

Results

(Age-specific) tetanus antitoxin immunity levels

In the nationwide sample 14.0% had tetanus antitoxin levels of less than 0.01 IU/ml (Table 1). A tetanus antitoxin level from 0.01 to 0.1 IU/ml was observed in 13.6% (95% confidence interval (CI) 12.5% to 14.7%), from 0.1 to 1 IU/ml, in 37.1% (CI 35.2% to 39.0%); from 1 to 10 IU/ml, in 33.6% (95% CI 31.3% to 35.9%); and at least 10 IU/ml, in 1.8% (95 % CI 1.4% to 2.2%). The tetanus antitoxin antibody level was higher for men; both the prevalence of tetanus antitoxin levels less than 0.01 IU/ml and the GMT were statistically significantly different between men and women (Table 1).

Table 1. Prevalence of tetanus antitoxin levels and GMT by gender in the nationwide sample; Pienter Project 1995-1996, The Netherlands

	Number	<0.01 IU/ml	(95% CI)	GMT	(95% CI)
Nationwide sample					
Total	7715	14.0	(12.9 - 15.1)	0.29	(0.26 - 0.32)
Men	3644	8.8	(7.9 - 9.7)	0.45	(0.41 - 0.50)
Women	4071	18.4	(16.8 - 20.1)	0.19	(0.17 - 0.22)

The prevalence of tetanus antitoxin levels varied by age (Figure 1, Tables 2 and 3). Less than 2% of those aged up to 35 years and less than 5% of those aged up to 45 years had tetanus antitoxin levels less than 0.01 IU/ml, while this percentage increased to 19.5% in 45 to 49-year-olds to 54.6% in 75 to 79-year-olds. This trend was reflected in the GMT value, which was greater than 0.4 IU/ml for 1 to 44-year-olds and was 0.10 IU/ml or less for 45 to 79-year-olds. Table 2 show elevated tetanus antitoxin levels and GMTs at the ages of one year, 4 years and 9 years. Analysis by age in months showed a slightly higher GMT for 6 months-olds and a peak in the GMT for 12-14-months-olds (data not shown). Furthermore, a not statistically significantly higher GMT was observed for 25 to 29-year-olds, particularly for men (Table 3). The GMTs were lower for those older than 29 years.

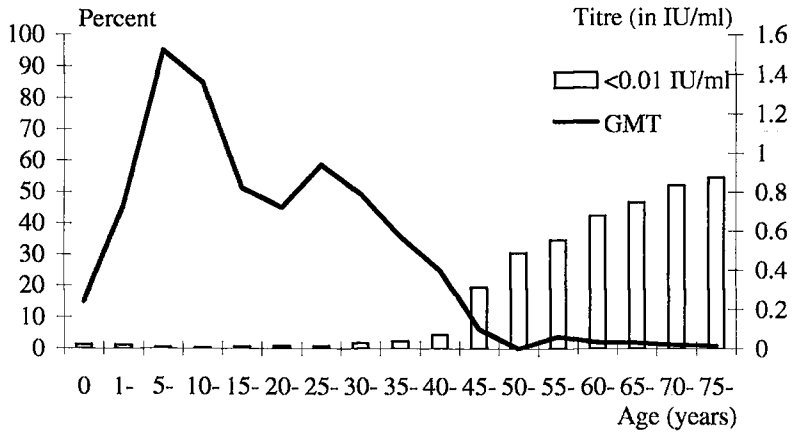

Figure 1. Age-specific prevalence of immunity to tetanus in the nationwide sample for 0 to 79-year-olds; Pienter Project, 1995-1996, The Netherlands

Table 2. Age-specific prevalence of tetanus antitoxin levels and GMT by gender in the nationwide sample for 0 to 14-years-olds; Pienter Project, 1995-1996, The Netherlands

Age group (years)	Number	<0.01 IU/ml	(95% CI)	GMT	(95% CI)
Men					
0	104	0.7	(0.0 - 2.0)	0.25	(0.19 - 0.32)
1	99	1.7	(0.0 - 3.8)	0.91	(0.70 - 1.19)
2	83	0.8	(0.0 - 2.3)	0.36	(0.28 - 0.47)
3	113	0.0	(-)	0.40	(0.28 - 0.57)
4	81	0.0	(-)	3.28	(2.17 - 4.96)
5	49	0.0	(-)	2.10	(1.47 - 3.0)
6	66	0.0	(-)	1.41	(1.02 - 1.93)
7	53	1.9	(0.0 - 5.9)	0.75	(0.54 - 1.05)
8	71	0.0	(-)	1.33	(0.92 - 1.90)
9	57	0.0	(-)	3.42	(2.24 - 5.22)
10	51	1.1	(0.0 - 3.4)	1.91	(1.39 - 2.64)
11	61	0.7	(0.0 - 2.2)	1.64	(1.28 - 2.10)
12	50	0.0	(-)	1.72	(1.25 - 2.36)
13	64	0.0	(-)	1.13	(0.90 - 1.41)
14	54	0.0	(-)	1.20	(0.92 - 1.57)
Women					
0	83	1.7	(0.0 - 5.1)	0.23	(0.15 - 0.33)
1	86	0.0	(-)	0.79	(0.53 - 1.19)
2	73	0.7	(0.0 - 2.1)	0.35	(0.26 - 0.47)
3	102	2.0	(0.0 - 4.3)	0.37	(0.25 - 0.56)
4	72	0.9	(0.0 - 2.6)	3.08	(1.99 - 4.78)
5	53	0.0	(-)	1.84	(1.26 - 2.68)
6	55	1.1	(0.0 - 3.4)	1.45	(1.00 - 2.11)
7	48	1.2	(0.0 - 3.6)	0.55	(0.40 - 0.76)
8	56	0.0	(-)	1.14	(0.77 - 1.71)
9	40	0.0	(-)	4.93	(3.54 - 6.87)
10	62	0.0	(-)	2.41	(1.84 - 3.16)
11	50	0.0	(-)	1.47	(1.12 - 1.93)
12	72	0.0	(-)	1.17	(0.96 - 1.43)
13	62	3.1	(0.0 - 9.5)	0.71	(0.46 - 1.10)
14	48	0.0	(-)	0.99	(0.77 - 1.28)

Table 3. Prevalence of tetanus antitoxin levels and GMT by age group (birth cohort) and gender in the nationwide sample for 0 to 79 years-olds; Pienter Project, 1995-1996, The Netherlands*

Age group (years)	Birth-cohort*	Number	<0.01 IU/ml	(95% CI)	GMT	(95% CI)
Men						
0	1995/96	104	0.7	(0.0 - 2.0)	0.25	(0.19 -0.32)
1-4	1991/95	376	1.1	(0.01 - 2.2)	0.75	(0.60 -0.95)
5-9	1986/90	296	0.3	(0.0 - 0.9)	1.61	(1.33 -1.94)
10-14	1981/85	280	0.4	(0.0 - 1.6)	1.46	(1.27- 1.68)
15-19	1976/80	209	1.0	(0.0 - 2.5)	0.93	(0.78 -1.10)
20-24	1971/75	139	0.3	(0.0 - 1.0)	0.93	(0.73- 1.20)
25-29	1966/70	150	1.4	(0.0 - 3.5)	1.35	(1.05- 1.73)
30-34	1961/65	188	2.5	(0.0 - 5.0)	1.21	(0.95- 1.55)
35-39	1956/60	220	1.8	(0.0 - 3.7)	0.86	(0.66 - 1.11)
40-44	1951/65	230	2.3	(0.0 - 4.7)	0.62	(0.49 - 0.80)
45-49	1946/50	208	10.4	(6.2 - 14.5)	0.24	(0.18 - 0.32)
50-54	1941/45	228	17.8	(11.9 - 23.7)	0.16	(0.11 - 0.22)
55-59	1936/40	251	12.6	(8.5 - 16.8)	0.21	(0.15 - 0.28)
60-64	1931/35	216	26.8	(20.5 - 33.2)	0.08	(0.05 - 0.11)
65-69	1926/30	200	31.0	(24.5 - 37.4)	0.07	(0.04 - 0.10)
70-74	1921/25	193	39.0	(30.5 - 47.5)	0.04	(0.03- 0.06)
75-79	1916/20	156	47.5	(37.4 - 57.5)	0.02	(0.01- 0.03)
Women						
0	1995/96	83	1.7	(0.0 - 5.1)	0.23	(0.16 - 0.33)
1-4	1991/95	333	1.4	(0.1 - 2.6)	0.68	(0.55 - 0.84)
5-9	1986/90	252	0.5	(0.0 - 1.3)	1.47	(1.22 - 1.77)
10-14	1981/85	294	0.6	(0.0 - 1.9)	1.29	(1.12 - 1.49)
15-19	1976/80	243	0.6	(0.0 - 1.9)	0.74	(0.63 - 0.87)
20-24	1971/75	199	1.0	(0.0 - 2.7)	0.60	(0.49 - 0.74)
25-29	1966/70	226	1.0	(0.0 - 2.5)	0.74	(0.59 - 0.93)
30-34	1961/65	244	1.2	(0.0 - 2.7)	0.59	(0.47 - 0.73)
35-39	1956/60	282	2.8	(0.0 - 5.7)	0.39	(0.31 - 0.48)
40-44	1951/65	247	6.2	(2.7 - 9.6)	0.26	(0.19 - 0.36)
45-49	1946/50	261	27.6	(20.0 - 35.3)	0.05	(0.03 - 0.07)
50-54	1941/45	264	42.6	(34.5 - 50.7)	0.03	(0.02 - 0.04)
55-59	1936/40	250	56.0	(49.3 - 62.6)	0.02	(0.01 - 0.02)
60-64	1931/35	237	55.5	(47.0 - 64.1)	0.02	(0.01 - 0.02)
65-69	1926/30	263	58.3	(51.2 - 65.4)	0.02	(0.01 - 0.02)
70-74	1921/25	218	62.4	(55.5 - 69.3)	0.01	(0.01 - 0.02)
75-79	1916/20	175	58.5	(49.3 - 67.6)	0.01	(0.01 - 0.02)

* Due to the data collection during more than 15 months overall 7% of the participants belonged to one younger birth cohort and 0.4% to one older birth cohort than the age group presented. The percentages and GMT with 95% confidence intervals were similar for the analyses by birth cohort and by age group.

Table 4. Frequency of self-reported and documented tetanus revaccination and participation in military service by gender in the nationwide sample for 0 to 79-year-olds; Pienter Project 1995-1996, The Netherlands

	Men (n = 3644)		Women (n = 4071)	
	Percent	(95% CI)	Percent	(95% CI)
Self-reported tetanus vaccination after an injury	42.6	(40.4 - 44.9)	29.5	(27.1 - 31.8)
Self-reported DT-IPV vaccination	54.7	(52.7 - 56.7)	49.7	(47.7 - 51.6)
Documented revaccination of tetanus	12.8	(10.8 - 14.8)	3.8	(2. - 4.8)
Military service history	33.2	(30.6 - 35.8)	0.1	(0.0 - 0.2)
Any indication of tetanus revaccination[a]	74.5	(72.6 - 76.4)	70.4	(68.6 - 72.3)

[a] Self-reported tetanus vaccination after an injury and/or self-reported DT-IPV vaccination and/or documented revaccination of tetanus and/or military service history

Table 5. Prevalence of tetanus antitoxin levels and GMT by gender among orthodox reformed individuals in the low vaccine coverage sample; Pienter Project 1995-1996, The Netherlands

	Number	<0.01 IU/ml	(95% CI)	GMT	(95% CI)
Orthodox reformed					
Total	233	37.4	(26.7 - 48.2)	0.05	(0.03 - 0.09)
Men	116	36.4	(29.1 - 43.6)	0.05	(0.02 - 0.10)
Women	117	42.2	(20.1 - 64.4)	0.06	(0.02 - 0.15)

The difference between men and women was largest for those at least 45 years of age but for all age groups the GMT was higher for men (Table 3). Men documented or reported tetanus vaccination more often (Table 4). When the analysis was restricted to those with documented tetanus revaccination, no statistically significant difference was found between men (GMT 0.89 IU/ml; 95% CI 0.70 to 1.14) and women (GMT 0.57; 95% CI 0.35 to 0.92). The difference between men and women remained when the analysis was restricted to individuals without documented revaccinations and when the analysis was restricted to those without documented or self-reported revaccinations.

Among orthodox reformed individuals the percentage of tetanus antitoxin levels below 0.01 IU/ml and GMT were higher than in the nationwide sample (Table 5). The percentage of orthodox

reformed individuals with tetanus antitoxin levels below 0.01 IU/ml was 40.7% (95% CI 26.6% to 54.8%) for 0 to 19-year-olds, 26.2% (95% CI 8.7% to 43.6%) for 20 to 39-year-olds and 46.4% (95% CI 33.0 to 59.8%) for 40 to 79-year-olds.

Among 0 to 19-year-olds 38.0% (95% CI 17.8% to 58.2%) and among 20 to 39-year-olds 28.5% (95 CI 9.3% to 47.7%) reported to have participated in the national immunisation programme. None of these orthodox reformed individuals had tetanus antitoxin levels below 0.01 IU/ml. For those who reported not to have participated in the national immunisation programme the percentage of individuals with tetanus antitoxin levels below 0.01 IU/ml amounted to 59.5% (95% CI 41.1% to 77.9%) for 0 to 19-year-olds and to 42.4% (95% CI 11.8% to 73.0%) for 20 to 39-year-olds.
The percentage of tetanus antitoxin levels below 0.01 IU/ml was somewhat, but not statistically significantly lower for men than for women. This difference was due to a lower percentage of tetanus antitoxin levels in men aged 20 to 39 years compared to women in this age group and remained when the analysis was restricted to individuals without self-reported vaccinations.
When orthodox reformed individuals were excluded from the low vaccine coverage sample, the GMT (0.32 IU/ml; 95% CI 0.26 to 0.39 IU/ml) and percentage of tetanus antitoxin levels below 0.01 IU/ml (16.5%; 95% CI 13.3% to 19.6%) were not statistically significantly different from those in the nationwide sample. The GMT for orthodox reformed individuals with a tetanus antitoxin level of at least 0.01 IU/ml was 0.23 IU/ml (0.14 to 0.39 IU/ml), lower than the GMT of 0.47 IU/ml (0.42 to 0.52 IU/ml) of those with a level of minimally 0.01 IU/ml in the nationwide sample.

Predictors of tetanus antitoxin levels

As no independent association of country of birth with tetanus antibody levels was found in the logistic regression analyses for those born outside the Netherlands, those born in Morocco or Turkey excluded, these individuals were pooled with individuals born in the Netherlands. Independent predictors of a tetanus antitoxin level below 0.01 IU/ml were: i.) lack of evidence for tetanus immunisation (odds ratio (OR) 0.19 (95% CI 0.16 to 0.23) , ii.) orthodox religion (OR 0.06; 95% CI 0.03 to 0.15) , iii.) low degree versus no degree of urbanisation (OR 0.69; 95% CI 0.55 to 0.87) , iv.) no self-reported travel to Asia (OR 0.38; 95% CI 0.28 to 0.52), to South America (OR 0.60; 95% CI 0.38 to 0.94) or to Central or South Africa (OR 0.61; 95% CI 0.39 to 0.96) v.) born in Morocco (OR 0.17; 95% CI 0.06 to 0.51) versus born in the Netherlands or other country. The OR for those born in Turkey amounted to 0.40 (95% CI 0.13 to 1.24) and was only statistically significant in a model without the variable 'evidence of tetanus vaccination'. Evidence of tetanus vaccination might be an intermediate variable in the association with other variables and tetanus antitoxin level. In a model without this variable the effects of religion, self-

reported travel to South America, Asia and Central or South Africa and country of birth were slightly stronger. No independent association with tetanus antibody levels was found for marital status, educational level, region, self-reported travel to Eastern Europe, Turkey or Greece, Middle East, Central America or North Africa.

Persistence of tetanus antitoxin antibodies

For individuals aged 10 to 34 years with the sixth and last documented tetanus vaccination at the age of 8 or 9 years (n=635) the GMT decreased from 1.38 IU/ml (95% CI 1.23 to 1.55 IU/ml) for 10 to 14 year-olds to 0.44 IU/ml (95% CI 0.29 to 0.68 IU/ml) for 30 to 34-year-olds (Figure 2). In linear regression analysis with the ^{2}log tetanus antitoxin titre, the intercept was -2.6 and the parameter estimate for the inverse of age in years amounted to 36.0. No effect for gender was observed. One individual (0.09%) had a tetanus antibody level below 0.01 IU/ml. The percentage of individuals with a level between 0.01 and 0.1 IU/ml was 3.2% (95% CI 0.0% to 6.5%) and ranged from 0.4% (10 to 14-year-olds) to 5.1% (20 to 24-year-olds). The GMT for individuals with documented revaccination (n = 39) was 1.88 IU/ml (95% CI 1.49 to 2.37 IU/ml). None of these individuals had a tetanus antitoxin level below 0.1 IU/ml. The median number of revaccinations was one (range 1 to 3). Due to the small numbers, no conclusion could be drawn from the number of revaccinations.

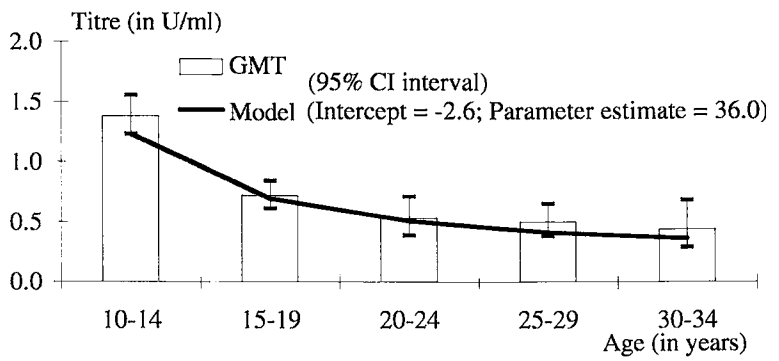

Figure 2. Tetanus antitoxin titre (GMT) by age group and linear regression of tetanus antitoxin antibody titre (in 2 log) with the reciprocal of age (in yrs) for individuals with the sixth and last documented vaccination at the age of 8 or 9 years (n=635); Pienter Project, 1995-1996, The Netherlands

Discussion

Unlike vaccination for other vaccine-preventable diseases such as diphtheria and poliomyelitis, tetanus vaccination does not indirectly protect other individuals (1). An important exception, the passive antibodies of newborns, offers us an opportunity to diminish the burden of neonatal tetanus in developing countries by vaccinating girls and women (11). The lack of indirect protection, together with the ubiquity of tetanus spores, means that every individual without tetanus protection is at risk of tetanus.

Although there is no absolute protective level of the antitoxin, 0.01 IU/ml is considered the minimum protection level (1). The tetanus antitoxin levels we found in the Dutch population (14% below 0.01 IU/ml, 13.6% between 0.01 and 0.1 IU/ml and 72.5% above 0.1 IU/ml) are more favourable than in most other studies (12-17).
Our results show that the Dutch immunisation programme has induced very good protection against tetanus. The percentage of individuals with tetanus antitoxin levels above the minimum protection level was very high (≥95%) for those born after its introduction in 1952 (i.e. aged less than 45 years in our study). For those who could have participated in the catch-up campaign (born between 1945 and 1952), this percentage was still high (80%).
When our data were interpreted longitudinally, like others, we found that tetanus immunity persisted for at least two decades, and probably even longer, in individuals who had received six vaccinations (i.e. they had been completely vaccinated in the Dutch immunisation programme) (1).
The regression analysis predicts a decline according to a hyperbolic curve in tetanus antitoxin level after complete participation in the Dutch vaccination programme. It is impossible to tell from these cross-sectional data whether age has an independent effect in addition to the time after the last vaccination. When a similar slope for the overall time/age effect is assumed outside the observed age range of 10 to 34 years, the regression analysis predicts a GMT of 0.22 IU/ml and a probability of an antibody level below the minimum protective level of less than 0.0001 at the age of 90 years, i.e. about 80 years after the sixth vaccination. In Danish studies a hyperbolic or exponential decline in antibody titres after primary vaccination with three doses was found (18-20). Higher levels were found in our study, which is consistent with a higher degree and longer duration of immunity with an increasing number of doses (1). After the moments of the third to sixth vaccination, the peak in GMT in each case was higher, and although the antitoxin level decreased sharply afterwards, the levels remained higher than before these moments. The Dutch vaccine contains 5 Lf tetanus toxoid per dose, which is comparable to the potency of the vaccine used in the United States, but 40 percent less potent than reported for the studies in Denmark (12,20). The longer duration of protective immunity after three tetanus immunisations in the

Danish study compared to a study in the United States might be associated with the higher vaccine potency (12,20).
Longitudinal interpretation of cross-sectional data like ours has been found to be reliable (21). One difficulty is that we had to rely on self-reported revaccination in our study. Thus, forgotten revaccinations could have led to a slower decrease in antitoxin level (21). However, if a faster, i.e. exponential, decline is assumed, the predicted GMT is 0.064 IU/ml and the probability of a antibody level below the minimum protective level does not exceed 0.14. Furthermore, we think that the self-reported revaccination of those individuals who brought documentation of all six vaccinations of the vaccination programme is more reliable than that of other individuals, and especially more so than that of those born before the introduction of vaccination.

The percentage of individuals with tetanus antitoxin levels above the minimum protective level decreased sharply for those born before the introduction of vaccination, i.e. above 50 years. It was less than 50% for those aged 75 to 79 years, and about 25% had an antitoxin level just above this minimum level (between 0.01 and 0.1 IU/ml). The higher percentage at risk of a tetanus infection in these age groups explains that of the 34 tetanus patients reported in 1984 to 1996, 30 patients (88%) were born before 1945. Only one patient, born in 1930, had been vaccinated during childhood. For two of the four tetanus patients born after 1945, the vaccination status was unknown, and the other two were not vaccinated. This pattern is consistent with other reports (12-14,17,22-28).

We found higher tetanus antitoxin levels for men. In other studies, these higher immunity levels were frequently ascribed to vaccinations given in military service, and they were sometimes thought to be due to higher accident rates (12,14-17,22,23,28,29). However, some found similar levels for men and women despite booster vaccinations in military service (13,30).
In our study the difference between men and women was largest for those aged at least 45 years, but for all age groups the GMT was higher for men. Although more men than women had documented or self-reported revaccination, a real difference between men and women could not be excluded. Higher antitoxin levels were found for men without documented or self-reported revaccination than for women. However, the difference might be explained by the unreliability of self-reported vaccination, especially for those born before or just after the introduction of the vaccination programme (16,21,24,25). Some of the revaccinated men might have forgotten that they had been revaccinated. For example, the higher tetanus antitoxin levels for men born between 1946 and 1950 compared to women in these cohorts, probably reflects a lower vaccine coverage in the first years after the introduction of the vaccination programme. In contrast to women, men who were not reached by the vaccination programme, could have received a primary serie of tetanus vaccinations in military service. Furthermore, no information was available on the number of revaccinations. In the regression model that was restricted to those with documented

vaccinations and without documented or self-reported revaccination, no gender effect was observed.

The lower antitoxin levels in the sample of municipalities with low vaccine coverage were due to the lower antitoxin levels for orthodox reformed individuals. It is remarkable that, in spite of the fact that these individuals frequently refuse vaccination more than 60% had antitoxin levels above the minimum protective level. Indeed, among 0 to 19-year-olds 38% and among 20 to 39-year-olds 29% reported to have participated in the national immunisation programme and all of these orthodox reformed individuals had antitoxin levels above the minimum protective level. However, the percentage of orthodox reformed individuals with antitoxin levels below the minimum protective level was higher in the youngest age group than in those aged 20 to 39 year-olds. Besides, also among orthodox reformed individuals who reported not to have participated in the national immunisation programme tetanus antitoxin levels above the minimum protective level were found.

Furthermore, although the estimated percentages of tetanus antitoxin levels below 0.01 IU/ml in the nationwide sample were hardly affected by selective nonparticipation, the estimated percentage of tetanus antitoxin levels below 0.01 IU/ml for the small subgroup of orthodox reformed individuals were probably somewhat underestimated. Orthodox reformed individuals who reported that they had participated in the vaccination programme were somewhat overrepresented among the participants. Nonparticipants for whom questionnaire data was available reported less frequently that they had participated in the vaccination programme compared to participants (results not shown). As no information on religion was available for nonparticipants who did not answer the questionnaire (29%), the true nonparticipation bias is unknown.

Orthodox reformed individuals with antitoxin levels above the minimum protective level had lower GMTs than those individuals in the nationwide sample. This suggests that they received fewer vaccinations than others. Perhaps these religious groups consider vaccination against tetanus after an injury not to be preventive, but therapeutic. Therapeutic interference is accepted by these religious groups. Also the not statistically significantly higher tetanus antitoxin levels for men might be explained -as described for the nationwide sample- by the fact that some men have received tetanus vaccinations in military service but did not report this.

Thus, it is impossible to obtain reliable insight into the acceptance of vaccination in this group and whether this acceptance has changed over time. However, as tetanus antibodies are only induced after vaccination, it is clear that a part of the orthodox reformed individuals were vaccinated. Since the Netherlands pursue a non-mandatory vaccination policy, acceptance of vaccination will be mainly dependent on decision of the concerning individual.

In addition to age by sex and religion, we studied whether other variables were associated with tetanus immunity. The lack of association of educational level, marital status and geographical

region suggests that the vaccination programme reaches different parts of the population equally well. However, we could not explain the somewhat higher risk of a tetanus antitoxin level below the protective level in municipalities with a low degree of urbanisation. The vaccine coverage in these municipalities for at least three DTP-IPV doses by the age of 12 months was even slightly higher than in the other municipalities (98.1% versus 97.2% in 1996) in the nationwide sample.
The greater risk of lower antibody levels for individuals born in Turkey or Morocco suggests that they had less vaccination coverage for tetanus. They account for the largest groups with non-Dutch nationality in our country. In a Canadian and American study, birth outside Canada and United States was found to be associated with lower tetanus protection (12,13). The difference might be due to varying vaccination schedules or varying administration of tetanus vaccinations in the countries.
The better protection of those who reported travel to Asia, South America, Central or South Africa than that of those travelling to Turkey or Greece, to the Middle East and to Eastern Europe might be due to the absence of guidelines for vaccination (for example in Greece) or to the shorter time since the advice has come into effect (Eastern Europe).

In conclusion, our results subscribe to the policy in our country in which no routine revaccination against tetanus is given. This contrasts with the policy in the United States and Canada. However, in the Netherlands the vaccine coverage is high and six doses are given in childhood. These factors have to be taken into account in decisions on the policy in other countries. Giving four or five doses during childhood followed by booster dose in adolescence might be as effective as our vaccination schedule in which the last and sixth vaccination is given at nine years of age. According to Dutch guidelines, tetanus revaccination for travel or profession is advised when the last vaccination was given at least 15 years ago. This advice could explain the peak in the GMT for 25 to 29-year-olds, i.e. 15 to 19 years after the last routine vaccination in the Dutch vaccination programme. The interval for revaccination after injury is 1 year. Although proper wound care and adequate tetanus prophylaxis after injury will have contributed to the very low incidence of tetanus, the high vaccine coverage in connection with the long persistence of tetanus immunity for those who completed the Dutch immunisation programme must have been very important too. After all, tetanus also develops in wounds so small that no medical care is sought and thus no tetanus vaccination is given. The long persistence of tetanus immunity suggests that our country would still err on the safe side by adhering to the WHO guidelines, which prescribe revaccination after injury only when the last vaccination is more than 5 years old.
A considerable number of adults born before the introduction of mass vaccination lack protective tetanus antibodies, and almost all tetanus cases were observed in these age groups. Since the Dutch immunisation programme induces long-term protection, we agree with Gergen et al. that offering a primary tetanus vaccination to cohorts born before the introduction of vaccination would probably be more effective in preventing tetanus than routine revaccination, e.g. every 10

years (12). Vaccination of these groups could be prove particularly efficient if combined with other vaccines (e.g. diphtheria).

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Chapter 6

Immunity against poliomyelitis in the Netherlands

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Abstract

With a view to eradicating polio insight into poliomyelitis immunity is useful, particularly when there are pockets of unvaccinated persons in the population. In 1995-1996 in a population-based study in the Netherlands, neutralizing antibodies against poliovirus types 1, 2, and 3 were measured in 9,274 sera of the general population and of sociogeographically clustered religious groups rejecting vaccination. In the general population, the antibody prevalence (≥1:8) was 96.6 (95 percent confidence interval (CI) 95.9 to 97.2 percent), 93.4 (92.3 to 94.5 percent) and 89.7 percent (88.3 to 91.0 percent) for poliovirus types 1, 2, and 3, respectively. Antibody prevalence was greater than 90 percent for type 1 and greater than 80 percent for types 2 and 3 for all age groups. Antibodies persist for long periods in both persons with natural immunity and persons with immunity induced by inactivated polio vaccine. Among orthodox reformed individuals, antibody prevalence of poliovirus types 1, 2, and 3 was 65.0 (57.2 to 72.9 percent), 59.0 (40.1 to 77.9 percent) and 68.7 percent (65.2 to 72.2 percent), respectively. For orthodox reformed participants, but not for other participants, the seroprofiles showed an effect from recent poliomyelitis outbreaks among orthodox reformed groups. Since the Netherlands pursues non-mandatory vaccination, global poliovirus eradication is the only way to protect these orthodox reformed groups.

In the 10 years since the World Health Organization launched the initiative to eradicate poliomyelitis by the year 2000, major progress has been made worldwide (1). As a result of increased global vaccination coverage, the reported incidence of poliomyelitis has dropped from over 35,000 cases in 1988 to approximately 5,000 in 1997 (2-4). Surveillance plays a major role in directing vaccination efforts and in the process toward eventually certifying that the world is free of poliovirus. The surveillance is based on registration and examination of clinical cases compatible with poliovirus infection. Although immunosurveillance is not required by the World Health Organization, it will provide insight into the protective immunity of (sub)populations. Immunosurveillance will be of added value, particularly in countries where there are pockets of unvaccinated persons, for polio eradication (5-7).

Poliovirus vaccination was introduced in the Netherlands in 1957. Children are vaccinated at the ages of 3, 4, 5, and 11 months with diphtheria, tetanus, pertussis, and inactivated polio vaccine (DTP-IPV) and at ages 4 and 9 years with DT-IPV. The coverage for at least three vaccinations at the age of 12 months has been 97 percent in the past decades (8,9). Despite this extensive vaccination coverage, there have been a number of poliomyelitis outbreaks in recent decades. These outbreaks were confined to orthodox reformed individuals who refuse vaccination on religious grounds. The last outbreaks occurred in 1978 with 110 reported cases, caused by poliovirus type 1, and in 1992-1993 with 71 cases, caused by poliovirus type 3 (10,11). The majority of unvaccinated persons in the Netherlands have not been vaccinated for various, mostly trivial reasons, but are protected because of natural immunity or herd immunity. An orthodox reformed minority of approximately 275,000 persons is however, insufficiently protected by herd immunity, since they form a sociogeographically closely knit network (12). It should be noted that vaccination is accepted to some degree even among the orthodox reformed group. About a third of the orthodox reformed participants in our study reported to have indeed received vaccination. This matches with the presence of tetanus antitoxin antibodies that we measured in the sera of this cohort (13).

We established a serum bank through a population-based sampling in 1995-1996 to evaluate the effects of mass vaccination in the Netherlands (7). This offered us an opportunity to study the prevalence of antibodies against poliovirus in the Dutch population and in groups refusing vaccination. The objective was to gain insight into the population's immunity. In addition, such data could provide insight into the possible waning of natural and vaccine-induced immunity in the absence of boosting opportunities. Furthermore, the study enabled us to look for indications of poliovirus circulation by comparing the serological profiles of cohorts born before and after recent outbreaks, both in the general population and in orthodox reformed groups.

Material and methods

Study population

Eight municipalities with probabilities proportional to their population sizes were sampled within each of five geographical Dutch regions with similar population sizes. An age-stratified sample (classes 0, 1-4, 5-9, ..., 75-79 years) of 380 individuals was randomly selected from each municipality. Subjects were requested to give a blood sample, fill out a questionnaire, and bring their certificates of national immunisation programme, military service, and travel vaccinations. In addition, individuals were selected similarly from eight municipalities with low vaccine coverage (65-87 percent) so that immunity in the orthodox reformed persons who refuse vaccination could be assessed. Samples and data were collected in the period from October 1995 to December 1996. The participation rates were 55 percent in the nationwide sample and 52.5 percent in the low vaccine coverage sample. The study design and details on the data collection have been published elsewhere (7).

Antibody assay

The sera were stored at minus 86 °C. Neutralizing antibodies against poliovirus types 1, 2, and 3 were determined in a microneutralization assay with the Mahoney strain for poliovirus type 1, the MEF-1 strain for poliovirus type 2, and the Saukett strain for poliovirus type 3, as previously described (14). The sera were titrated in a twofold dilution range up to 1:4096. The results are expressed as ^{2}log reciprocal titers. The titers are expressed as the reciprocal of the greatest dilution showing complete neutralization of the cytopathic effect of 100 percent cell culture infection doses. A titer of 1:8 (^{2}log titer=3) is defined as indicating full protective immunity; a titer of 1:4 or 1:2 (^{2}log titer=1 or 2), basic protective immunity; and 1:1 (^{2}log titer=0), as no protective immunity.

Statistical analysis

Frequencies and geometric mean titers within each municipality were weighted by the proportion of the age group in the population. To produce national estimates, the weighted frequencies and geometric mean titers were averaged over the 40 municipalities (15). For the low vaccine coverage sample, the geometric mean titers were averaged weighting by the population size of the municipality. The effect of differential probabilities of response on both sample estimates was less than one standard error and was therefore ignored.

In the different analyses the following groups were distinguished:

1. All participants of the nationwide sample (NS; N = 7,773).
2. All participants of the low vaccine coverage sample (LVC; N= 1,501).
3. Participants who were not orthodox reformed, in the low vaccine coverage sample (NOR-LVC; N= 1,265)
4. Orthodox reformed participants of the low vaccine coverage sample who frequently refuse vaccination (OR-LVC; N = 236).
5. The subgroup of orthodox reformed participants of the low vaccine coverage sample who did not report to have participated in the national immunisation programme and had no documented vaccinations against poliomyelitis (UOR-LVC; N = 167).

Linear regression analysis was used to determine the persistence of poliovirus type 1, 2, and 3 antibodies after complete participation in the national immunisation programme. The association between poliovirus type 1, 2, and 3 antibody titers and age was studied in this analysis for individuals from the nationwide sample with the sixth documented vaccination at 8 to 9 years of age, for those without self-reported or documented revaccination or a military service history. Since the number of individuals over 34 years of age who met these criteria was very small, this analysis was restricted to those aged 10 to 34 years.

Participants over 16 years of age were asked through the questionnaire whether they had received any additional vaccination for diphtheria, tetanus, and poliomyelitis (DT-IPV) after their childhood vaccination because of military service, travel, or professional activities, for example. Those participants who reported such a vaccination were considered revaccinated. Documented revaccination was defined as any documented vaccination with inactivated polio vaccine given in addition to vaccinations documented on a certificate of the national immunisation programme.

Results

Protective immunity in the nationwide sample (NS)

The percentages of full, basic, and no protection against poliovirus types 1, 2 and 3 are given for the nationwide sample in Table 1. The percentage of full protection was greatest (97 percent) for poliovirus type 1; it was slightly lower for poliovirus type 2 (93 percent) and amounted to 90 percent for poliovirus type 3. The percentages of full protection against poliovirus type 2 and poliovirus type 3 were observed to be smaller than that against poliovirus type 1 in almost all age classes (Figure 1). The percentages of full protection are greatest for those up to 29 years of age for all serotypes. A gap in the percentage of full protection was observed in the age groups of 30 to 44 years for poliovirus type 3. The percentage of full protection for poliovirus type 3 in these

age groups ranged from 82.3 to 88.1 percent, while for poliovirus types 1 and 2 this percentage remained greater than 90 percent. In the groups over 49 years of age, the percentage of full protection ranged from 90.7 to 94.8 percent for poliovirus type 1, from 80.7 to 90.0 percent for poliovirus type 2, and from 82.3 to 91.3 percent for poliovirus type 3.

The geometric mean titer was lower for 0-year-olds than for 1 to 4-year-olds for all poliovirus types. For 1 to 14-year-olds, it remained stable for poliovirus types 1 and 2, but a slight increase was seen for poliovirus type 3. The geometric mean titers gradually decreased for those from 15 to 40 years of age for all poliovirus types (Figure 1). The geometric mean titers decreased from 10.3, 8.6, and 8.2 for 10 to 14-year-olds to 7.7, 6.2, and 5.2 for 35 to 39-year-olds for poliovirus types 1, 2, and 3. Thereafter, the geometric mean titers remained stable. The geometric mean titer was highest for poliovirus type 1 and lowest for poliovirus type 3 in all age groups. No differences were observed between men and women.

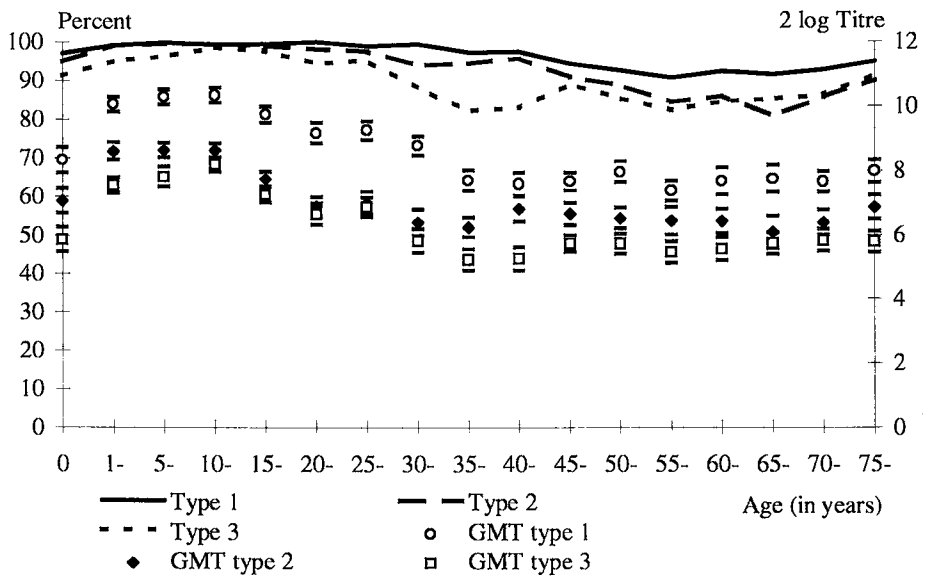

Figure 1. Age-specific prevalence of antibodies and geometric mean titer (with 95 percent confidence intervals) for poliovirus types 1, 2, and 3 in the nationwide sample (NS); Pienter Project, 1995-1996, The Netherlands

Table 1. The percentages of full, basic, and no protective antibodies and geometric mean titers for 0 to 79-year-olds for poliovirus types 1, 2, and 3 in the nationwide sample (NS), low vaccine coverage sample (LVC), and separately for orthodox reformed individuals from the low vaccine coverage sample (OR-LVC) and others from the low vaccine coverage sample (NOR-LVC); Pienter Project, 1995-1996, The Netherlands

	Nationwide sample (NS) (n = 7,773)		Low vaccine coverage sample (LVC) (n = 1,501)		Low vaccine coverage sample excluding orthodox reformed individuals (NOR-LVC) (n = 1,265)		Orthodox reformed individuals from the low vaccine coverage sample (OR-LVC) (n = 236)	
	%	(95% CI)	%	(95% CI)	%	(95% CI)	%	(95% CI)
Poliovirus type 1								
Full protection	96.6	(95.9 - 97.2)	92.3	(89.5 - 95.0)	97.2	(96.0 - 98.4)	65.0	(57.2 - 72.9)
Basic protection	2.3	(1.8 - 2.7)	3.5	(1.9 - 5.2)	1.6	(0.9 - 2.3)	12.5	(7.5 - 17.5)
No protection	1.2	(0.8 - 1.5)	4.2	(2.2 - 6.2)	1.2	(0.0 - 2.5)	22.5	(16.7 - 28.3)
Geometric mean titer	*8.6*	*(8.4 - 8.8)*	*8.4*	*(7.9 - 8.8)*	*8.9*	*(8.4 - 9.3)*	*5.6*	*(4.5 - 6.6)*
Poliovirus type 2								
Full protection	93.4	(92.3 - 94.5)	87.7	(85.1 - 90.3)	92.8	(91.4 - 94.1)	59.0	(40.1 - 77.9)
Basic protection	4.1	(3.4 - 4.8)	6.3	(4.9 - 7.7)	4.7	(3.8 - 5.5)	16.3	(2.3 - 30.3)
No protection	2.4	(1.8 - 3.1)	6.0	(3.7 - 8.2)	2.6	(1.8 - 3.3)	24.7	(17.8 - 31.6)
Geometric mean titer	*7.0*	*(6.8 - 7.2)*	*6.7*	*(6.5 - 6.9)*	*7.0*	*(6.8 - 7.3)*	*4.5*	*(3.2 - 5.9)*
Poliovirus type 3								
Full protection	89.7	(88.3 - 91.0)	85.9	(84.2 - 87.7)	89.8	(87.1 - 92.6)	68.7	(65.2 - 72.2)
Basic protection	7.1	(6.2 - 8.0)	7.0	(5.7 - 8.3)	5.5	(4.1 - 6.9)	14.2	(9.4 - 19.1)
No protection	3.2	(2.4 - 4.0)	7.1	(5.2 - 8.9)	4.7	(2.1 - 7.2)	17.1	(11.2 - 23.0)
Geometric mean titer	*6.3*	*(6.1 - 6.5)*	*6.2*	*(6.0 - 6.4)*	*6.6*	*(6.2 - 6.9)*	*4.8*	*(4.4 - 5.1)*

Persistence of poliovirus antibodies after vaccination in the nationwide sample (NS)

For individuals aged 10 to 34 years in the nationwide sample who had had the sixth and last documented vaccination at the age of 9 years, without any evidence of revaccinations (n = 969), the overall percentages of full, basic, and no protection in this group were 99.7 (95 percent CI 99.2 - 100), 0.06 (95 percent CI 0.0 - 0.18) and 0.2 percent (95 percent CI 0.0 -0.6) for poliovirus type 1, 98.7 (95 percent CI 97.7 - 99.6), 1.2 (95 percent CI 0.2-2.1) and 0.1 (95 percent CI 0.0 - 0.3) for poliovirus type 2 and 94.5 (95 percent CI 90.9 - 98.2), 5.1 (95 percent CI 1.5 - 8.8) and 0.3 percent (95 percent CI 0.0 - 0.7) for poliovirus type 3. The geometric mean titers for poliovirus type 1 decreased from 10.5 (95 percent CI 10.2 to 10.7) for 10 to 14-year-olds to 8.0 (95 percent CI 7.4 to 8.6) for 30 to 34-year-olds, for poliovirus type 2 from 8.6 (95 percent CI 8.4 to 8.8) to 5.2 (95 percent CI 4.7 to 5.7) and for poliovirus type 3 from 8.2 (95 percent CI 8.0 to 8.5) to 4.8 (95 percent CI 4.2 to 5.3) (Figure 2).
In the linear regression analysis of the relationship between antibody titers and age (Figure 2), the intercepts for poliovirus types 1, 2, and 3 were 11.9, 10.8, and 10.1 (in ^{2}log titer), respectively, with slopes for age in years of -0.12, -0.18, and -0.16 respectively.

For the 39 individuals who had received the sixth vaccination at the age of 8 or 9 years and had had at least one documented revaccination, the geometric mean titers averaged 10.4 (95 percent CI 9.7-11.1) for poliovirus type 1, 9.4 (95 percent CI 8.6 - 10.3) for poliovirus type 2, and 8.2 (95 percent CI 7.0 - 9.4) for poliovirus type 3. These geometric mean titers were similar to those of individuals aged 10 to 14 years who received the last and sixth vaccination at the age of 8 or 9 years.

Protective immunity in orthodox reformed individuals (OR-LVC)

A more detailed analysis was carried out on the low vaccine coverage sample. The orthodox reformed individuals within this sample (OR-LVC) were compared with others within this sample (NOR-LVC).
Orthodox reformed individuals showed a smaller percentage of full protection for all three poliovirus types than others in the low vaccine coverage sample, as well as those in the nationwide sample (Table 1). When orthodox reformed individuals were excluded from the low vaccine coverage sample (NOR-LVC), no differences were observed in full, basic, and no protection and geometric mean titers for poliovirus types 1, 2, and 3 between the low vaccine coverage sample and the nationwide sample (NS) (Table 1).

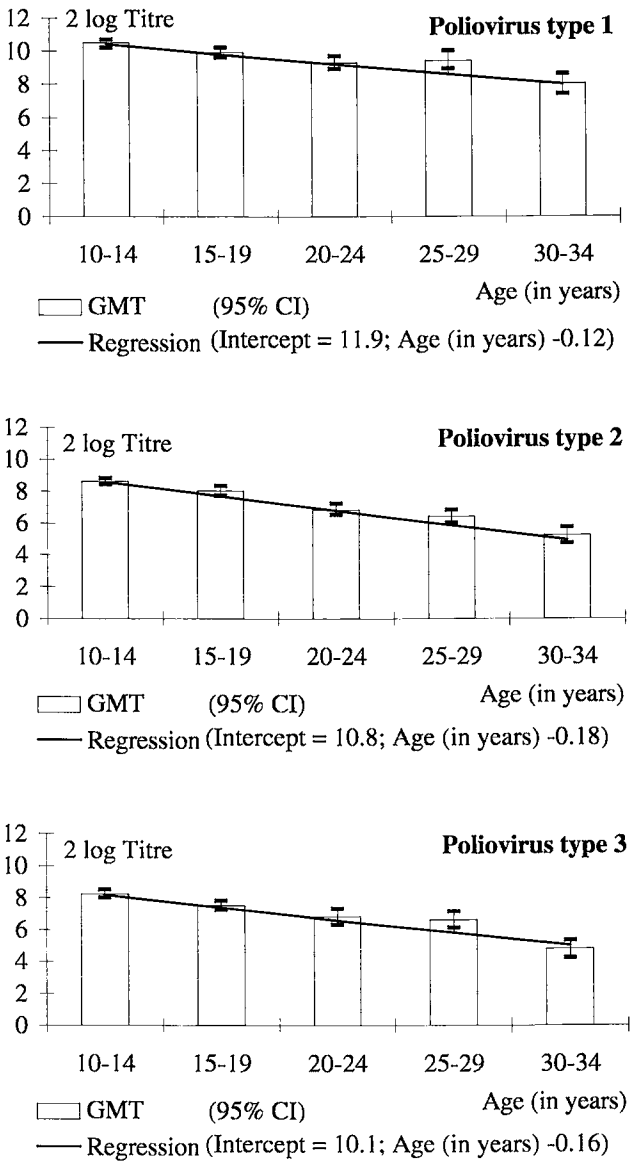

Figure 2. Regression analysis for poliovirus types 1 to 3 for individuals with the sixth poliomyelitis vaccination at the age of 8 or 9 years without self-reported or documented revaccination in the nationwide sample (NS); Pienter Project, 1995-1996, The Netherlands

The age-specific percentages of full protection for poliovirus types 1, 2, and 3 for the orthodox reformed individuals in the low vaccine coverage sample (OR-LVC) are compared with all individuals in the nationwide sample (NS) in Figure 3. Due to the small numbers of orthodox reformed individuals, the age-specific percentages are less precise; therefore the fluctuations shown could be a result of chance.
For poliovirus type 1, the percentage of full protection in the orthodox reformed individuals (OR-LVC) increases from 39.1 percent for 15 to 19-year-olds to 88.7 percent for 30 to 34-year-olds. This percentage is more or less stable at a level of approximately 90 percent for older individuals. An increase in the percentage of full protection for poliovirus type 2 is observed after the age of 25 to 29 years from 54.4 percent to a level of approximately 80 percent. The percentage of full protection increases after the 1 to 4-year-olds (54.2 percent) and then fluctuates around an average of 75 percent for poliovirus type 3.

Effects of recent outbreaks: cohortwise analysis

We did a cohortwise analysis of the serological profiles in order to study the possible effect of poliovirus circulation during recent outbreaks among orthodox reformed individuals, i.e., in 1978 and in 1992-1993. We compared the percentages of full and no protection in various birth cohorts in the nationwide sample (NS) for this purpose, in the low vaccine coverage sample excluding all orthodox reformed individuals (NOR-LVC) and in the orthodox reformed individuals who did not report to have participated in the national immunisation programme and had had no documented vaccinations against poliomyelitis (UOR-LVC) (Table 2). The following birth cohorts within these groups were distinguished: those born after 1992, i.e., after the epidemic with poliovirus type 3, those born between 1978 and 1992, i.e., after the epidemic with polio virus type 1 in 1978, those born between 1945 and 1978, i.e., those who had been offered childhood vaccination, and those born before 1945, i.e., those who were not eligible for childhood vaccination. Table 2 summarizes the results.

No statistical significant differences were found in the percentages of full protection for individuals in the nationwide sample and the not orthodox reformed individuals from the low vaccine coverage sample (NOR-LVC). However, the percentages of full protection were slightly smaller for those born between 1978 and 1992 and, in particular, those born after 1992 in the low vaccine coverage sample (NOR-LVC), than for those in the nationwide sample (NS). The difference was largest for poliovirus type 3, but was not statistically significant. In contrast, considering those born before 1945 the percentages of full protection for poliovirus types 1 and 3, were slightly larger for those in the low vaccine coverage sample (NOR-LVC) than for those in the nationwide sample (NS). There was little difference for type 2.

Table 2. The percentages of full and no protection against poliovirus types 1, 2, and 3 according to birth cohort in the nationwide sample (NS), the low vaccine coverage sample without orthodox reformed individuals (NOR-LVC), and in a subgroup of unvaccinated orthodox reformed individuals from the low vaccine coverage sample (UOR-LVC); Pienter Project, 1995-1996, The Netherlands

	Poliovirus type 1		Poliovirus type 2		Poliovirus type 3	
	%	(95% CI)	%	(95% CI)	%	(95% CI)
Nationwide sample (NS) (n = 7,773)						
Born before 1945	91.8	90.4 - 93.3	84.9	82.9 - 86.9	84.6	82.7 - 86.4
(n = 2,511)	(2.9)	(2.0 - 3.8)	(6.9)	(5.6 - 8.2)	(6.0)	(4.7 - 7.4)
Born between 1945 and	97.9	97.2 - 98.6	95.9	94.8 - 97.0	90.2	88.5 - 92.0
1978 (n = 2,956)	(0.5)	(0.2 - 0.9)	(0.9)	(0.3 - 1.4)	(2.6)	(1.7 - 3.5)
Born between 1978 and	99.4	98.9 - 99.9	99.2	98.5 - 99.9	97.1	96.1 - 98.1
1992 (n = 1,675)	(0.2)	(0.0 - 0.6)	(0.5)	(0.0 - 1.0)	(0.6)	(0.2 - 1.0)
Born after 1992	98.4	97.2 - 99.6	98.2	97.1 - 99.2	93.8	91.7 - 96.0
(n = 631)	(1.0)	(0.0 - 2.0)	(1.1)	(0.07 - 2.0)	(1.3)	(0.4 - 2.3)
Low vaccine coverage sample excluding orthodox reformed individuals (NOR-LVC) (n = 1,265)						
Born before 1945	94.0	91.6 - 96.4	85.2	83.3 - 87.1	87.5	83.5 - 91.5
(n = 431)	(2.0)	(0.4 - 3.5)	(7.7)	(5.8 - 9.7)	(6.7)	(4.0 - 9.5)
Born between 1945 and	98.0	96.8 - 99.1	94.3	91.9 - 96.7	90.1	85.7 - 94.4
1978 (n=499)	(0.8)	(0.0 - 1.7)	(1.1)	(0.0 - 2.4)	(4.4)	(0.8 - 8.1)
Born between 1978 and	98.3	94.8 - 100	98.3	94.8 - 100	94.8	91.4 - 98.2
1992 (n = 248)	(1.7)	(0.0 - 4.0)	(0.3)	(0 - 1.3)	(1.3)	(0.0 - 3.4)
Born after 1992	96.4	90.7 - 100	93.1	85.3 - 100	83.4	73.0- 93.8
(n = 87)	(3.0)	(0.0 - 8.5)	(3.6)	(0 - 9.2)	(8.6)	(0.6 - 16.7)

Table 2 continued

	Poliovirus type 1		Poliovirus type 2		Poliovirus type 3	
	%	(95% CI)	%	(95% CI)	%	(95% CI)
Orthodox reformed individuals (UOR-LVC) (n = 167)						
Born before 1945	86.9	76.0 - 97.8	70.0	47.2 - 92.8	61.3	25.7 - 96.9
(n = 60)	(8.8)	(0.0 - 20.3)	(4.1)	(0.0 - 12.8)	(19.9)	(0.0 - 55.2)
Born between 1945 and	64.0	50.2 - 77.9	60.0	33.2 - 86.1	54.3	31.3 - 77.4
1978 (n = 58)	(19.5)	(3.3- 35.7)	(20.1)	(0.0 - 42.8)	(28.7)	(12.3 - 45.1)
Born between 1978 and	12.1	0.0 - 24.4	18.8	0.0 - 42.4	52.8	8.1 - 97.4
1992 (n = 27)	(62.4)	(22.3 - 100)	(71.4)	(50.1 - 92.6)	(27.6)	(0.0 - 63.1)
Born after 1992	5.8	0.0 - 13.4	5.3	0.0 - 12.7	1.3	0.0 -5.8
(n = 22)	(31.5)	(0.0 - 65.5)	(75.2)	(53.8 - 96.6)	(78.8)	(51.8 - 100)

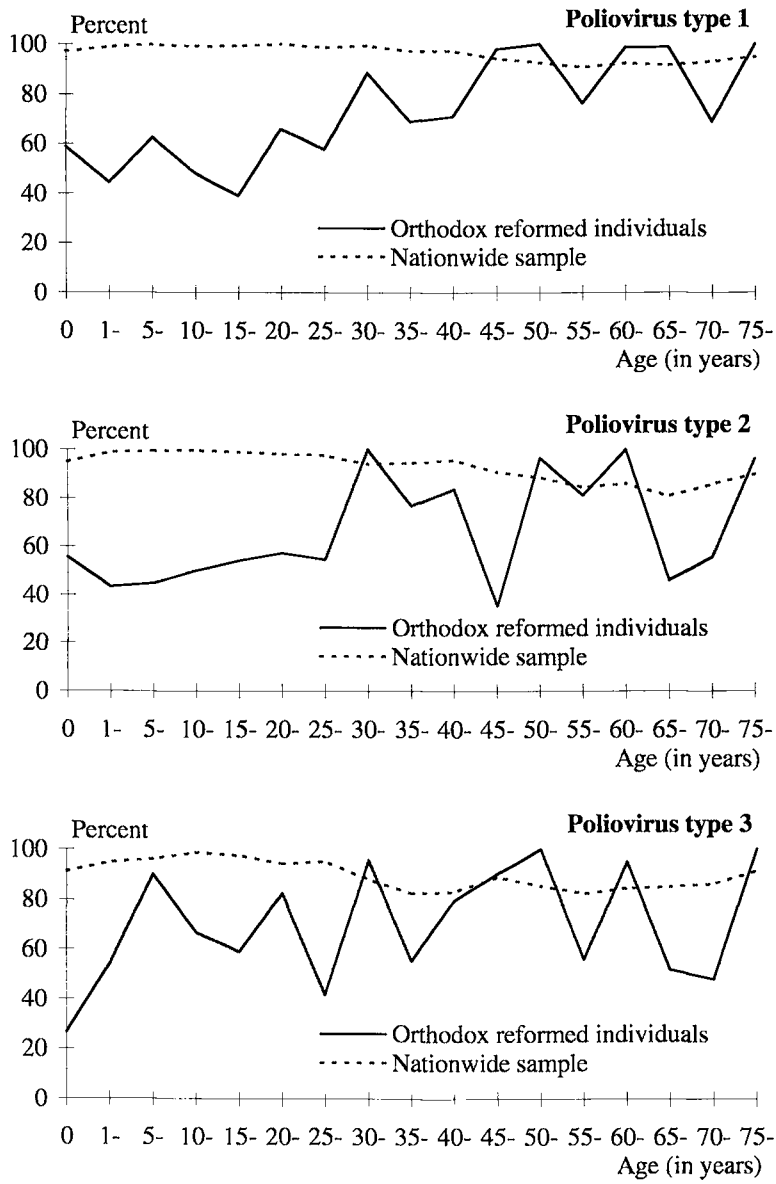

Figure 3. Age-specific prevalence of antibodies against poliovirus types 1, 2, and 3 in the nationwide sample (NS) and in orthodox reformed individuals from the low vaccine coverage sample (OR-LVC); Pienter Project, 1995-1996, The Netherlands

Within both the nationwide sample (NS) and the low vaccine coverage sample (NOR-LVC), the percentages of full protection were the smallest for those born before 1945 (with the exception of the smaller percentage for type 3 for those born after 1992 in the low vaccine coverage sample), while the greatest percentages were observed for those born between 1978 and 1992. By contrast, among the orthodox reformed individuals (UOR-LVC), the percentages of full protection for all serotypes were smallest in the cohorts born between 1978 and 1992 and after 1992. For these individuals, the difference between those born before and after the two epidemics was much larger than in the nationwide sample and the low vaccine coverage sample. Less than 20 percent of the orthodox reformed individuals born between 1978 and 1992 and less than 6 percent of those born after 1992 were fully protected against poliovirus types 1 and 2. In contrast, 53 percent of those born between 1978 and 1992 had full protective antibody levels against poliovirus type 3, as did only 1 percent of those born after 1992.
In all cohorts, whether born before or after the introduction of childhood vaccination (1945), the percentages of full protection for poliovirus types 1, 2, and 3 were smaller for orthodox reformed individuals than for individuals from the nationwide sample or the low vaccine coverage sample.

Discussion

Our study shows that the Dutch population is generally well protected against poliomyelitis. A large neutralizing antibody prevalence (≥ 1:8), often exceeding 90 percent, was found in all age groups. With the currently available methods, it is not possible to determine whether these antibodies are due to exposure to live, wild virus or to vaccine virus. In general, however, it is reasonable to assume that the neutralizing antibodies are mainly due to natural infection in those born before 1945, whereas they are mainly induced by vaccination with inactivated polio vaccine in those born after the start of childhood vaccination (1957).
Although the prevalence of antibodies (≥ 1:8) against poliovirus type 3 is generally somewhat lower than for types 1 and 2, it is still close to 90 percent. It can be assumed that even persons with a basic level of antibodies (1:4 and 1:2) are protected. It is, however, unclear at present whether all those without detectable antibodies are susceptible to infection; some, particularly the elderly, may be protected by memory immunity. We are currently performing a study of memory response in seronegative elderly persons after challenge with oral polio vaccine. Moreover, the potential for poliovirus circulation in the well-protected population vaccinated with inactivated polio vaccine is an important issue for polio eradication.

The age-specific profile of antibodies is mirrored in the age-specific geometric mean titers as shown in Figure 1. The geometric mean titers are consistently high. In the cohorts born before 1945 (i.e., in our study of those aged 50 years or more) with predominantly natural immunity, the geometric mean titers are stable without any indication of waning immunity. A seroprevalence

study with a different sampling scheme and limited numbers was carried out in the Netherlands in 1980 and 1985. Comparing the present results with those from the previous studies indicates that there is no waning immunity in these naturally infected persons several decades after natural infection (16,17). Naturally exposed individuals seem to keep their neutralizing antibodies. This would imply that those without detectable antibodies have never been exposed to live, wild virus. In contrast, a small decrease in the geometric mean titer with age or time was observed for those between 10 and 40 years old with probably predominantly vaccine-induced antibodies. It appears that the decrease in geometric mean titer is due to waning immunity after vaccination. No further decline was observed in the first cohorts to whom vaccination has been offered (40 to 49-year-olds). These cohorts are also likely to have been exposed to live polioviruses circulating during their childhood, and therefore probably have a combination of natural and vaccine-induced immunity. It is remarkable that the differences in antibody prevalence between poliovirus type 3 and types 1 and 2 are stronger in 30 to 44-year-olds. This might be due to a smaller immunogenicity of the poliovirus type-3 antigens. As such, assessment of poliovirus type-3 antibodies may provide the most sensitive tool to study possible waning immunity after vaccination.

In 10 to 34-year-olds who have been completely vaccinated according to the Dutch vaccination programme and do not have evidence of revaccination, regression analysis showed a linear decrease in antibody levels for all types. Yet antibody levels were still high, even about 20 years after vaccination. We cannot rule out the possibility that the long persistence of high levels of vaccine-induced antibodies is partly influenced by boosting through circulating poliovirus. This might correspond to the slightly smaller slope for poliovirus type 1 in the model, as poliovirus type 1 was most prevalent in the past. However, as we discuss later, virus circulation probably stopped in the late 1960s or early 1970s (17-19). In a follow-up study of children vaccinated with inactivated polio vaccine in Sweden, Böttiger et al. show that poliovirus neutralizing antibodies could persist for more than 18 years after vaccination (20). A more marked decline in antibody titer was seen in the first few years, while a very slow decrease was observed afterwards (20).

Epidemiological data of the recent polio outbreaks in the Netherlands confirm the high level of protective immunity against poliomyelitis in the general population. In both the 1978 and the 1992-1993 outbreaks, cases occurred only among unvaccinated individuals nearly all of whom belonged to orthodox reformed groups. Our results, however, show that there is still a high potential for outbreaks in these groups, since 23 percent, 25 percent, and 17 percent had no measurable antibodies for poliovirus type 1, type 2, and type 3, respectively. The percentage of susceptible individuals 1 to 19 years of age is 39 percent for type 1, 44 percent for type 2, and 19 percent for type 3. As expected, seronegative individuals are seen predominantly in the cohorts born after the introduction of vaccination. Actually, this seems to be a paradoxical effect of mass

vaccination, although these unvaccinated groups benefit from the interruption of virus circulation that results from widespread vaccination. The Ministry of Health, advised by the Health Council, has deliberately refrained from making vaccination mandatory in the Netherlands mainly for ethical reasons. Therefore, the ultimate prevention of poliomyelitis in these groups can only be attained through global eradication of the causative agents, the polioviruses (21).
The effect of virus circulation during the outbreaks in 1978 (type 1) and 1992-1993 (type 3) among orthodox reformed individuals without evidence of vaccination becomes evident when the serological profiles of the birth cohorts born before and after the outbreaks are compared. For type 1, the seroprevalence for those born between 1978 and 1992 was only 12 percent, but it was 64 percent for those born between 1945 and 1978; for type 3, the seroprevalence for those born after 1992 was only 1 percent in contrast to 53 percent for those born between 1978 and 1992. This indicates that as many as half of the individuals in these unvaccinated groups were infected during the epidemic. Remarkably, the cohortwise estimates of the seroprevalence for type 2 show the same pattern as for type 1, although there have been no signs of wild poliovirus type-2 circulation in the Netherlands for decades. The last polio patient with a poliovirus type-2 infection in the Netherlands was reported in 1966. Occasionally a wild poliovirus type 2 has been isolated from adopted children and, once, from river water in the early 1980s. Thereafter, only a few vaccine-derived poliovirus type-2 strains have been isolated (17). The apparently elevated poliovirus type-2 seroprevalence in those born after 1978 cannot be ascribed to vaccination with oral polio vaccine during the outbreak in 1978, as monovalent type-1 oral polio vaccine was used then to control the outbreak. In the 1992-1993 outbreak, however, trivalent oral polio vaccine was applied, which may have resulted in poliovirus type-2 circulation (22). There is some cross-reactivity between poliovirus serotype 1 and 2, but it is improbable that this can explain the comparable levels for serotypes 1 and 2 satisfactorily (23). Aylward et al. also conclude from sero-epidemiological data from a study in gypsies that wild virus circulation could have influenced the prevalence, although the possibility that the high prevalence was caused by the spread of vaccine virus could not be ruled out (24). The differences in prevalence in poliovirus type-3 antibodies in cohorts born before and after the 1992-1993 outbreak in our study must mainly be ascribed to wild virus circulation.

In the nationwide sample and the low vaccine coverage sample excluding orthodox reformed individuals we saw no differences in type-1 seroprevalence in the cohorts born before and after the 1978 outbreak, and there was only a small difference in type-3 seroprevalence in cohorts born before and after the 1992-1993 outbreak. This supports the assumption that there has been little or no virus spread outside the orthodox reformed groups, either in the general population or among other inhabitants of the municipalities where these groups live. In addition, the age-specific geometric mean titers for types 1, 2, and 3 in the nationwide and low vaccine coverage samples are very similar, which is another indication of the absence of poliovirus circulation in the general

population. We must realize that substantial virus circulation would have to occur to cause a significant difference. In those born after 1978 and particularly in those born after 1992 , the type-3 seroprevalence in the low vaccine coverage sample excluding orthodox reformed persons is lower than in the nationwide sample. The reason for this is unclear, but might be explained by the study results being influenced by other religious groups who live in these municipalities, some whom, as is known, also refuse vaccination, be it less strictly.

The level of protective immunity of individuals in the orthodox reformed groups without evidence of vaccination who were born before the introduction of childhood vaccination is lower than that of other persons of the same age. As we expect them to have been exposed to the same extent, it is likely that some of the elderly outside the orthodox reformed groups have been vaccinated for various other reasons, i.e., travel, military service, professional activities. The same has been observed for the prevalence of tetanus antitoxin antibodies (13).

As we have discussed, there are no signs of waning immunity in cohorts supposed to have predominantly natural immunity. The seroprevalence for none of the three types reaches 100 percent in the oldest cohorts, indicating that the endemic virus in those days never fully depleted the pool of susceptible individuals. This is in agreement with the threshold value for the percentage of protected persons required to prevent polio virus transmission, which is estimated at 82-87 percent under the condition of homogeneous mixing (25). The 1978 and 1992-1993 outbreaks also appear not to have infected all susceptible individuals among the orthodox reformed groups; only 64 percent of those born between 1945 and 1978 have type-1 antibodies, and 53 percent of those born between 1978 and 1992-1993 have type-3 antibodies. The large percentage of children without measurable antibodies clearly shows a potential for another polio outbreak in the Netherlands that will exist as long as polioviruses have not been eradicated worldwide.

Conclusions

Routine childhood vaccination with inactivated polio vaccine has provided excellent protection against poliomyelitis in the general population in the Netherlands. Antibodies persist for very long periods, not only in naturally infected persons, but also in persons with vaccine-induced immunity. Our study, in the era of polio eradication provides additional evidence of absence of poliovirus circulation in the general population during the outbreaks that occurred among orthodox reformed groups who refuse vaccination in 1978 and 1992-1993. Since the Netherlands pursues a nonmandatory vaccination policy, pockets of susceptibility remain because of their objecting to vaccination for religious reasons. Global eradication, therefore, would be the means of protecting these individuals against poliomyelitis.

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Part 3

Surveillance and seroepidemiology of pertussis

Chapter 7

Pertussis in the Netherlands: an outbreak despite high levels of immunisation with whole-cell vaccine

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Abstract

In 1996, a sudden increase in pertussis incidence was reported in the Netherlands (2.1 per 100,000 in 1995, 18 per 100,000 in 1996). Although not all potential surveillance artifacts could be excluded, it is highly probable that the data reflect a true outbreak. However, the cause of this increase has not yet been determined. Further research is directed to the severity of disease and a possible mismatch between the vaccine and the circulating Bordetella strains.

In 1996, 2771 cases were reported to the Inspectorate of Health in the Netherlands (population 15 million), compared with 319 cases in 1995. With epidemic cycles expected every 3 to 5 years and a recent outbreak in 1994, this rise was unexpected (1).

After the introduction of pertussis immunisation with a whole-cell vaccine in the National Immunisation Programme (1952), the incidence of pertussis in the Netherlands decreased significantly. Children are immunized at the age of 3, 4, 5, and 11 months with a diphtheria, tetanus, pertussis, inactivated polio vaccine (DTP-IPV), and the vaccine coverage for pertussis, for at least three immunisation amounts to 96% at the age of 12 months. Until the 1980s, the incidence of pertussis seemed very low, because only incidentally cases were reported. However, in the last two decades pertussis has been endemic and epidemic peaks have appeared.

The surveillance of pertussis is mainly based on notification, which was made obligatory by law in 1976. Because the availability and interpretation of serologic tests changed in the 1980s and the absence of a clear case definition for notification seemed to have influenced the surveillance, a restrictive case definition that included criteria for laboratory diagnosis, was introduced in 1988. Therefore, we have limited this report to the notification data from 1989 onwards. The annual incidence of pertussis notification from 1989 to 1995 is shown in Figure 1. In this period, the case definition includes defined clinical symptoms and laboratory confirmation. The clinical symptoms include a serious cough lasting more than 2 weeks or cough attacks or cough followed by vomiting in combination with at least one of the following symptoms/findings: apnea, cyanosis, characteristic cough with whooping, subconjunctival bleeding, leukocytosis, or contact with a confirmed or suspected pertussis in the previous 3 weeks. Laboratory confirmation is defined as either positive culture of *Bordetella pertussis* or *Bordetella parapertussis*, or positive two-point serology, i.e., the finding of a significant rise (> 4-fold) of IgG antibodies against pertussis toxine and/or IgA antibodies against *B. pertussis* in paired sera. The case definition for notification is highly specific, but results in significant underreporting.

We compared the number of cases reported per 4-week period in 1996 with the number reported in 1994, the year with the highest incidence in recent years (Figure 2). It appears that an unexpectedly large number of pertussis cases was reported (18 per 100,000) in 1996.
The reports came from all over the country. No geographical clustering was observed, even in regions with pockets of low vaccination coverage or at the borders of the country (close to Germany, where vaccination coverage for pertussis is low).
The age distribution of the patients in the years 1989 to 1996 shows a significant decrease in the proportion of infants less than 1 year old (from 21% in 1989 to 7% in 1996) and a significant increase in the proportion of 1- to 4-year-olds (from 21% in 1989 to 30% in 1996). A significant decrease (from 42% in 1989 to 30% in 1995) was also observed in 5- to 9-year-old children;

however, in 1996, the proportion increased to 39%. No changes were observed for 10- to 14- and 15- to19-year-old groups, whereas the proportion of patients 20 years old or older increased from 5% to 11% (Table 1).

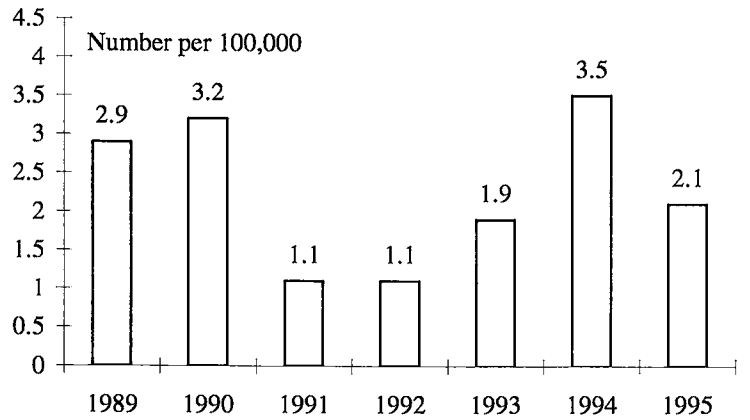

Figure 1. Annual incidence of pertussis estimated from notification by registraton date for the period 1989-1995

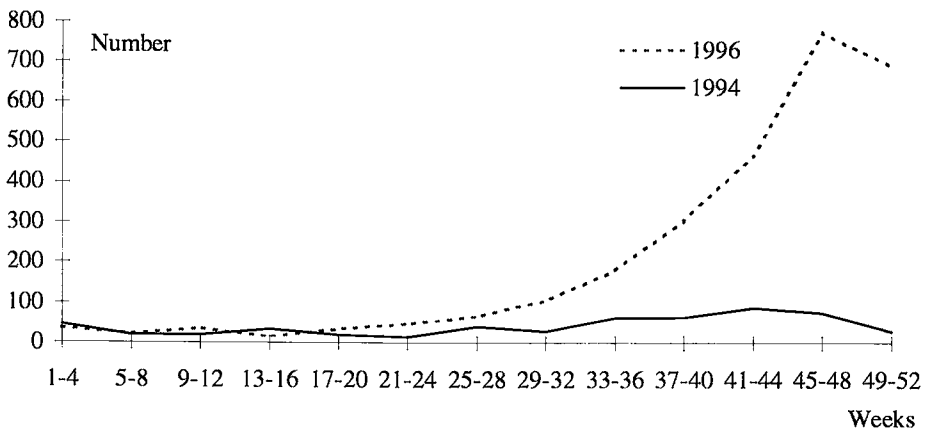

Figure 2. Number of cases notified by registration date in 1994 and 1996 (4-week periods)

Table 1. Age distribution (percentages) pertussis notification in the period 1989-1996

Age (years)	1989	1990	1991	1992	1993	1994	1995	1996
0	21	21	23	21	24	16	13	7
1-4	21	21	26	19	27	34	33	30
5-9	42	37	34	30	26	32	30	39
10-14	10	9	10	17	12	8	10	11
15-19	1	2	2	2	1	1	1	2
≥ 20	5	9	6	11	10	8	12	11
Unknown	0	0	1	1	0	1	1	0
Total (n)	434	471	164	169	294	536	319	2771

Whereas the average vaccine coverage did not change, the proportion of vaccinated patients increased from 55% in 1989 to 85% in 1996. In all age groups, except that of 6- to 11-months old infants, the proportion of vaccinated patients is higher in the years 1994 to 1996 than in the years 1989 to 1993.

We estimated the annual incidence of pertussis for vaccinated and unvaccinated children aged 1 to 4 and 5 to 9 years, on the basis of reporting by registration date (1989 to 1996) (Figure 3). Calculation of incidence according to vaccination status was deliberately restricted to those aged 1 to 4 years and 5 to 9 years (vaccine coverage estimated at 96%) for methodological reasons. The changes in age-specific incidence over the years for vaccinated and unvaccinated children are identical for 1989 to 1993. However, from 1994 to 1996, and particularly in 1996, the incidence among vaccinated 1- to 4-year-olds was considerably higher than in 1989 and 1990. In unvaccinated children, the incidence in 1994 and 1995 was lower than in 1989 and 1990. The difference in incidence between the period 1989 to 1990 and the year 1996 was much smaller in unvaccinated than in vaccinated persons. A similar discrepancy can be seen for 5- to 9-year-old group.

In view of the increase in pertussis reporting in 1996, the first issue to be addressed was whether this reporting reflected a true increase in pertussis incidence or was a surveillance artifact. If considerable underreporting is assumed, an increase due to improved compliance to the notification system or increased alertness should be considered. However, in the first two quarters of 1996, there is no apparent reason for such an assumption. Nonetheless, after the first reports of the observed rise, both publicity and increased awareness of the disease by physicians and patients might have influenced reporting.

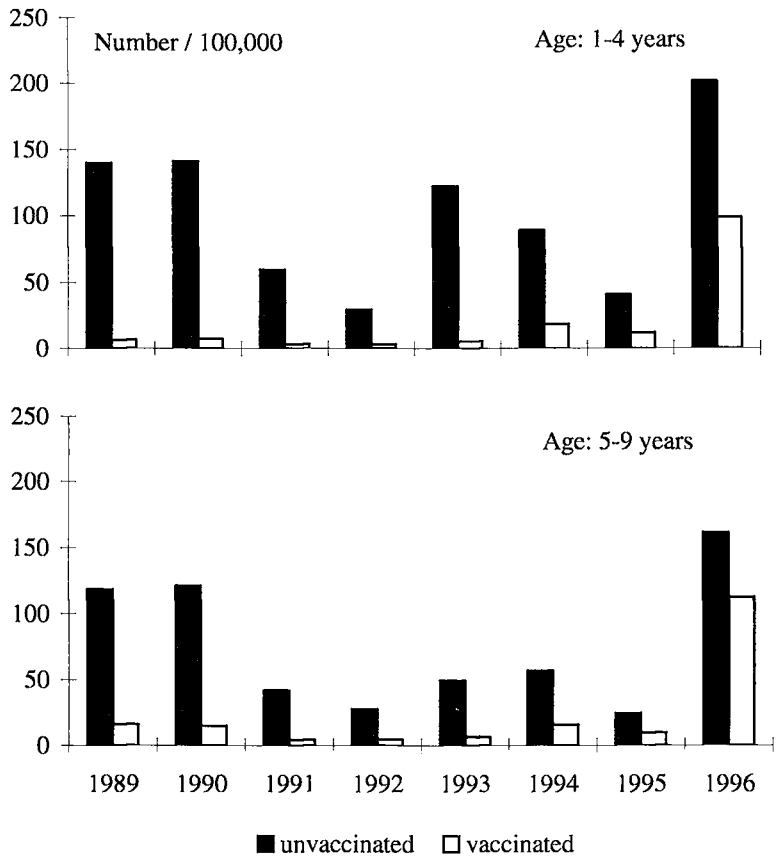

Figure 3. Estimated incidence of pertussis for unvaccinated and vaccinated children aged 1-4 years and 5-9 years based on notification records and assuming a coverage of 96% for the entire population

Serodiagnosis for the country as a whole is done by a single reference laboratory at the National Institute of Public Health and the Environment; thus, the data provided by the laboratory can be used for nationwide surveillance. Furthermore, the reference laboratory collects strains of *B. pertussis* from regional public health laboratories, and the number of strains received also reflects the epidemic curve. The epidemiologic curve derived from these various sources over the past years is consistent with the notification data.

Since laboratory confirmation is part of the case definition, changes in serodiagnostic practice might have influenced notification. Frequently, no conclusive results are obtained from serology: a second serum sample may not be submitted, or the first serum sample may be taken late after onset of

disease, thereby missing the dynamic phase of the immunoresponse. Recently, cut-off values for antibody titres characteristic for the acute immunoresponse after natural infection with *B. pertussis* have been determined by comparing the titre distribution in the healthy population, in recently vaccinated children, and in sera longitudinally collected after natural infection (2). Since 1993, high titres in acute-phase sera are reported to be "suggestive for recent infection with *Bordetella pertussis*". It can be imagined that such cases are reported, albeit not strictly in accordance with the case definition.

To exclude the possibility that the rise in notifications was the result of reporting cases diagnosed with positive one-point serology only, we linked the notification database and the serodiagnosis database. (The data were taken from January 1993 to September 1996). Special permission to link the databases was given by the Inspectorate of Health. It appeared that some notification was based on positive one-point serology as early as 1993, and this proportion increased from 20% in 1993 to 33% in 1994. It remained at this level in the subsequent years. Thus, the 1996 increase in notification cannot be attributed to this change in serodiagnostic practice.

Our surveillance data suggest a decrease in vaccine efficacy, but estimation of vaccine efficacy from surveillance data should be interpreted with caution. However, there are no indications of significant biases resulting from physicians' perceptions of vaccine efficacy that could have caused selective reporting of vaccinated patients; a higher probability of a positive serologic test result due to priming in vaccinated persons; or misclassification of cases with respect to vaccination status.

Although not all potential surveillance artifacts could be excluded, it is highly probable that the surveillance data reflect a true pertussis outbreak.

The outbreak can be caused by (1) decrease in vaccination coverage, (2) decrease in vaccine quality, (3) interference with other vaccines, (4) changes in circulating strains of *B. pertussis* that are not covered by vaccine-induced immunity, or (5) a combination of these factors.

A decrease in vaccination coverage has not been observed in the (accurate) registration system. The whole-cell vaccine used was produced in the National Institute of Public Health and the Environment and meets international standards. There was no sign of a gradual deterioration of the vaccine quality as determined for product release using the mouse protection test.

In April 1993, vaccination against *Haemophilus influenzae* type b (Hib) was added to the immunisation programme. The polyribosylribitolphosphate tetanus toxoid conjugated vaccine (PRP-T) was administered simultaneously with the DTP-IPV, although on different limbs. Interference of Hib vaccination with the immunoresponse to pertussis vaccine has been described (3). In a prospective serologic study on the potential interference in the Netherlands, this effect was not observed (4). Furthermore, the increase of pertussis in 5- to 9-year-old vaccinated children, who never received Hib vaccination, was similar to the increase in the 1- to 4-year-old

age group. Decreased vaccine efficacy due to interference of Hib vaccination is, therefore, very unlikely.
Molecular typing of *B. pertussis* isolates collected since 1945 indicated a shift in population structure, possibly due to the introduction of whole-cell vaccine (5). Further, antigenic variants of *B. pertussis* distinct from the strains incorporated in the whole-cell vaccine appear to have emerged (6). It might be possible that circulating strains of *B. pertussis* have become less sensitive to vaccine-induced immunity. The abrupt increase in the proportion of vaccinated patients reported in all age groups suggests such a change.

Data from other countries in Europe indicate that the pertussis epidemic is restricted to the Netherlands. However, a resurgence of pertussis has been noted in the United States since the late 1980s and in Canada since 1991. No single factor has been found to explain this resurgence (7-10).

The decrease in pertussis notification in the Netherlands at the end of 1996 may indicate a seasonal variation in the incidence. The 1996 data, including those on hospital admissions, are still being analyzed. These data will give insight into the severity of the pertussis epidemic among young infants. Protection of these infants is the main reason for pertussis vaccination. In January 1997 active (monthly) surveillance by pediatricians of cases among hospitalized children was added to the routine surveillance based on notification and laboratory surveillance.
A prospective study is being considered to assess efficacy of the whole-cell vaccine, including differentiation of severity of illness and number of immunisations received, if the outbreak continues. Cooperative studies to determine the distribution of restriction fragment length polymorphism types and antigenic variants of *B. pertussis* in various countries in Europe, Asia and North-America are underway. Also, the immunogenicity of the Dutch whole-cell vaccine is being assessed: whether it has changed over time and how it compares to published immunogenicity data of whole-cell vaccines with known, prospectively determined, vaccine efficacy (11).

In conclusion, in the Netherlands a sudden increase of pertussis notification has been observed, which seems to reflect a true increase in incidence. Nevertheless, the cause of this increase has not been definitively determined. A possible mismatch between the vaccine and the circulating Bordetella strains is being investigated.

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Chapter 8

Re-emergence of pertussis in the Netherlands: observations on surveillance data

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Abstract

Pertussis notifications, data about deaths, hospital admissions, and positive serodiagnoses from 1976-1998 were analyzed to help explain the Dutch pertussis outbreak in 1996. The unexpected outbreak in 1996 (as it appeared from notifications) was also observed in the other surveillance sources. In 1996, according to notifications and serology data, the increase in pertussis incidence among (mostly unvaccinated) children less than 1 year of age was similar to the increase in hospital admissions. In older, mostly vaccinated, individuals the increase of hospital admissions was relatively small. The increase of reported vaccinated patients of all ages was larger than that of unvaccinated patients. We postulate that the proportion of pertussis infections resulting in recognizable symptoms has increased among vaccinated individuals due to the mismatch of the vaccine strain and circulating *Bordetella pertussis* strains. Perhaps the small immunogenicity profile of the Dutch vaccine, has resulted in greater vulnerability to antigenic changes in *B. pertussis*.

Introduction

Introduction of mass vaccination against pertussis has greatly reduced pertussis incidence, but, even in countries with high vaccination coverage, pertussis resurgence has been described (1-4). We reported a sudden increase in pertussis notifications in the Netherlands in 1996 that reflected a true outbreak (5). The outbreak could not be explained by a decrease in vaccination coverage, which remained stable at 96% for at least three vaccinations in the first year of life. The vaccine meets international standards. No sign of an abrupt or gradual deterioration of vaccine quality, as determined at product release by the mouse protection test, was found. No interference of the introduction of vaccination against *Haemophilus influenzae* type b in 1993 with the immunoresponse to pertussis was found (6). No cohort effect in children vaccinated for *H. influenzae* type b was observed either (5). Perhaps a mismatch between the vaccine and circulating *Bordetella pertussis* strains contributed to the re-emergence (5,7-9).
Insight into pertussis epidemiology based on notification data is hampered by changes in case definition for notification, availability of laboratory diagnostic tests, interpretation thereof (mainly of serology), notification rate, and diagnostic practice. Therefore, we compare data from various surveillance sources, i.e., data regarding deaths, hospital admissions, and positive serodiagnosis with notification data from January 1976 to September 1998. This will be helpful in obtaining insight into pertussis epidemiology and provides possible explanations for the pertussis epidemic.

Methods

Surveillance data

Notification data

Data from pertussis notifications, which were made obligatory by law in 1976, were obtained for the period 1976 to 1998 from the Inspectorate of Health. In the period 1976 to 1987, there was no case definition for notification. In 1988, a case definition was introduced; it includes clinical symptoms and laboratory confirmation (or close contact with a laboratory-confirmed pertussis case).
Since the introduction of the case definition the clinical symptoms are a serious cough, lasting more than two weeks, coughing attacks, or coughing followed by vomiting in combination with at least one of the following symptoms/findings: apnea, cyanosis, characteristic cough with whooping, subconjunctival bleeding, or leucocytosis.

From 1988 to April 1997, laboratory confirmation was defined as either a positive culture of *B. pertussis* or *B. parapertussis*, or positive two-point serology. Two-point serology was considered

positive if a significant rise of IgG antibodies against pertussis toxin and/or IgA antibodies against *B. pertussis* in paired sera was found.
In April 1997, the Inspectorate of Health decided that, in addition to these laboratory results, a positive polymerase chain reaction (PCR) and positive one-point serology were acceptable as laboratory confirmation. Positive one-point serology was defined as high IgG and/or IgA antibody titers in a single serum sample as described under the heading *Pertussis serology.*

For the period 1976 to 1988, only aggregated data were available regarding certain points. They are: the total number of patients per year based on the notification date, the number of vaccinated (at least three vaccinations) and unvaccinated or incompletely vaccinated (0 to 2 vaccinations) patients and the number of patients with unknown vaccination status with age at notification in age groups (0, 1-4, 5-9 and ≥ 10 years). For the period 1989 to 1992, the date of notification and age in years at notification were available on an individual level in a database, while the aggregated data on vaccination status was similar to that for the period 1976 to 1988. Only since 1993 have the following data been included in the database: the date of onset of symptoms, date of birth, age in years at notification, vaccination status, method of laboratory diagnosis, and whether there had been contact with a laboratory confirmed case (i.e., epidemiologically confirmed cases). The method of laboratory diagnosis was differentiated as "microbiological" (positive culture or PCR), "serological", "epidemiological" (i.e., contact with a laboratory-confirmed case), and "unknown".

As a consequence of the available data, the case distribution over the years 1976 to 1988 could only be assessed by date of notification. For the period 1989 to 1992, the date of onset of symptoms was estimated by finding the median duration between the first day of illness and the date of notification for patients in the period 1993 to 1994. This median duration (81 days) was then substracted from the notification date of the case at hand. The distribution of cases over the years 1989 to 1992 was based on this estimate, while it was based on the date of first symptoms available in the database for the period 1993 to September 1998. For 1989 to 1992, the age distribution was based on the age at notification, while for 1993 to September 1998, it was based on the age at onset of symptoms.

Hospital admissions

The number of hospital admissions with pertussis as the main diagnosis (ICD-9-CM 033) in the years 1976 to 1997 were obtained from the registry of the Foundation Information Center for Health Care. For the period 1989 to 1997, data by age group (0, 1-4, 5-9, 10-14, 15-19, ≥ 20 years) were available.

Pertussis deaths

The number of deaths by age in 5-year classes due to pertussis in the period 1976 to 1997 were obtained from the Central Bureau of Statistics.

Pertussis serology

Data on pertussis serology were obtained from our National Institute of Public Health and the Environment. From the introduction of routine serology in the period 1982 to 1 January 1998, this was the only laboratory in the Netherlands that performed pertussis serology for patients with suspected whooping cough. The few other laboratories that also perform pertussis serology since 1998 are estimated to cover 10 to 15% of pertussis serology of the population.

Serology consisted of measuring IgA antibodies against a crude cell-wall preparation of *B. pertussis* (available since 1981) and IgG antibodies against purified pertussis toxin (available since 1984) in ELISAs according to previously described methods (10,11). The interpretation of serology has varied over the years. In the period 1982 to 1988, mostly single sera were submitted. Since it had been established that vaccination with the Dutch whole cell vaccin only induced low levels of IgG against pertussis toxin (IgG-PT) and no IgA against *B.pertussis* (IgA-Bp), detection of IgA-Bp or moderate, high or very high IgG-PT was reported as "supportive for pertussis". Around 1987 it became clear that low and moderate IgG-PT and IgA-Bp levels were present in a large proportion of the population and that the prevalence increased with age. Therefore, in 1988 the interpretation of serology in single sera was abandoned; paired sera were requested and only the finding of a significant increase of IgG-PT and/or IgA-Bp in such paired sera was reported to confirm the diagnosis of pertussis. As mentioned earlier, at the same time point the strict case definition for notification was introduced and "positive two-point serology" was included in the laboratory confirmation criteria of that case definition. However, in 1993 sera from the population, vaccinees, and patients with pertussis were tested. It was shown that high IgG-PT and/or high IgA-Bp levels greater than a (age-specific) cut-off value were very rare in the population (<2.5%). Such levels were not induced by vaccination, were present in the majority of patients with PCR- or culture-confirmed pertussis and decreased again within 6 months to levels below that cut-off value (12,13). Since 1994 the laboratory reported detection of such high values in a single serum or in both sera of a serum pair to indicate "possible pertussis" although the case definition for notification remained unchanged. From April 1997, the detection of such high levels in sera from patients was formally defined as "positive one-point serology" and included in the case definition for notification as being acceptable as laboratory confirmation of pertussis.

We were able to retrieve from the serological database patient data of patients with the day of onset in the period January 1989 to September 1998 and of patients with date of serology result registered in the period January 1986 to December 1987. Patients with positive two-point serology in the period 1989 to 1998 and patients with positive one-point serology in the period 1994 to 1998 were selected. Furthermore, the criteria we recently defined for positive one-point serology were retrospectively applied to the serological data of the periods 1986 to 1987 and 1989 to 1993. The distribution of cases in 1986 and 1987 was based on the year of the test result and, in the period 1989 to 1998, on the year of first symptoms.

Data analysis

We used the screening method and assumed an average vaccine coverage in the Dutch population of 96% to estimate the vaccine efficacy for those aged 1 to 4 and 5 to 9 years with notification data from 1976 to 1997. We compared completely vaccinated individuals (at least three vaccinations) with incompletely vaccinated or unvaccinated individuals (less than three vaccinations) (14).

For the period 1993 to 1997, the notification database and the database with records of serodiagnosis were linked on the level of individual patients to verify on which type of serodiagnosis (positive two-point serology or positive one-point serology) the notification was based. The coverage of the notification system was calculated from the proportions of reported patients with positive two-point serology and with positive one-point serology. We used the statistical package SAS to analyze the data. We calculated the vaccine efficacy in EPI INFO version 6.04.

Results

Pertussis notifications from 1976 to September 1998

In the first years after the introduction of notification by law, the reported number of cases was smallest (Table 1). From 1983 to 1987, that is, after immunoassays for pertussis serology became available, the number of reported patients rose yearly. In 1988, the year in which a case definition for notification was introduced and positive serology was restricted to the finding of an increase in titer in paired sera, the reported number of cases declined sharply. Between 1989 and 1995 somewhat greater numbers of cases were reported in the periods 1989-1990 and 1993-1994. In 1996, the number of cases was 12-times the number in 1995, while a twofold decrease of the 1996 number was observed in 1997.

The number of notifications from January to September 1998 (N = 1582) was smaller than that of the same periods in 1996 (N = 2171) and 1997 (N = 2004), but about six times larger than the average number of notifications in the same months of the years 1989 to 1995 (N = 269).

Comparison with hospital admissions

Although the trend for hospital admissions was similar to that of notifications, the ratios of notifications to hospital admissions varied for various periods (Table 1). This ratio was below 1 from 1976 to 1984, rose to 5.7 in 1987, and decreased sharply to 1.2 in 1988. It remained relatively stable from 1989 to 1995, but increased to 8.2 in 1996 and 6.1 in 1997.

Comparison with positive serology

Positive one-point serology

The cases with positive one-point serology followed a trend similar to that of the notifications in the years 1986 to 1987 and 1989 to 1998, for which data were available (Table 1). In 1986 and 1987, the ratio of notifications to cases with positive one-point serology was highest (0.8); it decreased in 1989, and remained relatively stable from 1989 to 1996 (0.2 to 0.4). This proportion increased in the period 1996 to 1998 from 0.5 to 0.7.

Positive two-point serology

The trend for cases with positive two-point serology and the trend in notifications were comparable (Table 1). From 1989 to 1995, the number of cases were similar (ratio 0.7 to 1.5), according to both notifications and positive two-point serology, while in 1996 and 1997 (ratio 2.2 and 2.9) and particularly in 1998 (ratio 5.4) the number of notifications was greater than the number of cases with positive two-point serology.

Comparison with deaths

In the period 1976 to 1997, seven deaths due to pertussis were registered: one in 1981, two in 1993, two in 1996, and two in 1997. They occurred among children less than 1 year old with the exception of one death in 1993 in the age group of 5 to 9 years.

Table 1. Absolute numbers of pertussis notifications, hospital admissions, cases with positive two-point serology, cases with positive one-point serology and ratio of notifications to hospital admissions in the period 1976 to 1997

Year		Notifications	Hospital admissions	Positive two-point serology	Positive one-point serology	Ratio of notifications and hospital admissions	Ratio of notifications and positive two-point serology	Ratio of notifications and positive one-point serology
1976	Introduction of notification law	4	52	*	*	0.1	*	*
1977		25	93	*	*	0.3	*	*
1978		1	32	*	*	0.0	*	*
1979		26	43	*	*	0.6	*	*
1980		30	65	*	*	0.5	*	*
1981	Introduction of IgA-assay	50	76	*	*	0.7	*	*
1982		80	98	*	*	0.8	*	*
1983		200	223	*	*	0.9	*	*
1984	Introduction of IgG-assay	534	200	*	*	2.7	*	*
1985		1522	313	*	*	4.9	*	*
1986		2159	388	*	2717	5.6	*	0.8
1987		2709	474	*	3295	5.7	*	0.8
1988	Case definition for notification (including strict criteria for interpretation of serology)	112	92	*	*	1.2	*	*

Table 1 continued

Year		Notifications	Hospital admissions	Positive two-point serology	Positive one-point serology	Ratio of notifications and hospital admissions	Ratio of notifications and positive two-point serology	Ratio of notifications and positive one-point serology
1989		523	221	350	1760	2.4	1.5	0.3
1990		397	157	278	1204	2.5	1.4	0.3
1991		145	82	110	411	1.8	1.3	0.4
1992		160	101	238	861	1.6	0.7	0.2
1993		346	288	482	1489	1.2	0.7	0.2
1994		519	276	498	1867	1.9	1.0	0.3
1995		341	162	272	1070	2.1	1.3	0.3
1996		4231	513	1885	7854	8.2	2.2	0.5
1997	Formal acceptance of positive one-point serology	2671	436	924	4107	6.1	2.9	0.7
1998§		1582§	*	294§	2187§	*	5.4	0.7

* Not available

§ Data from January to September 1998

Age-specific incidence according to notifications from 1989 to 1997

In the period 1989 to 1995, the average annual incidence according to notifications was greatest for infants less than 1 year old. Notification data for the years 1993 to 1995 show that the age-specific peak incidence occurred among 0 to 5-month-olds (Table 2). For infants less than 1 year old, according to notifications, the incidence in 1996 is a fourfold of the average incidence from 1989 to 1995, while it shows at least a 13-fold increase for older age groups. The age-specific peak incidence shifted to 4-year-olds in 1996 and 1997.

Notifications stratified by method of diagnosis

In the period 1993 to 1997, for which the method of laboratory diagnosis was available for the notifications, similar shifts in age distribution were observed for those cases confirmed by microbiological method, for cases with positive two-point serology and for cases with positive one-point serology. However, reported cases confirmed microbiologically (i.e., culture and/or PCR) were those of the relatively youngest patients, and cases confirmed with one-point serology were those of the oldest.

Comparison of notifications with hospital admissions

In contrast to notification data, the greatest age-specific incidence of hospital admissions occurred among infants less than 1 year old from 1989 to 1997 (Table 2). The increase in incidence in 1996 was more stable for the various age groups.

Comparison of notifications with positive serology

Positive two-point serology
Similarly to notifications, as a result of a relatively greater increase among older age groups, the peak incidence occurred among 0 to 5-month-olds from 1989 to 1995 and among 4-years-olds in 1996 and 1997 (Table 2).

Positive one-point serology
The incidence of cases with positive one-point serology was greatest among 7-year-olds in 1989 and it shifted towards 4-year-olds from 1992 to 1997. Like notification data, the increase for infants less than 1 year old was smaller (fourfold) than that of older age groups (fivefold to sevenfold) (Table 2).

Table 2. Average annual age-specific incidence (I, number per 100,000) and average proportional age distribution (%) in the period 1989-1995, in 1996 and in 1997

	Notifications						Hospital admissions					
Period	1989-1995		1996		1997		1989-1995		1996		1997	
	I	%	*I*	%	*I*	%	*I*	%	*I*	%	*I*	%
Age (years)												
0	36.9	20.7	160.4	7.3	110.4	7.9	66.2	68.2	184.0	68.4	150.9	65.8
0-5 m	*57.3*	*14.3*	*219.2*	*5.0*	*125.1*	*4.5*	--	--	--	--	--	--
6-11 m	*22.2*	*5.4*	*101.7*	*2.3*	*95.7*	*3.4*	--	--	--	--	--	--
1-4	12.0	26.2	152.8	28.7	90.1	26.5	4.9	21.2	11.6	17.9	12.4	22.2
5-9	12.6	32.8	162.5	37.2	84.0	30.9	1.3	6.8	4.9	9.2	3.4	7.6
10-14	3.8	10.5	57.4	12.3	33.0	11.3	0.4	2.5	1.2	2.1	0.5	1.1
15-19	0.5	1.4	10.3	2.3	8.4	2.9	0.01	0.2	0.3	0.6	0	0.0
≥ 20	0.3	8.4	4.4	12.2	4.6	20.5	0.02	1.1	0.08	1.8	0.1	3.2
Total	2.3	100	27.3	100	17.1	100	1.2	100	3.3	100	2.8	100

	Positive two-point serology						Positive one-point serology					
Period	1989-1995		1996		1997		1989-1995		1996		1997	
	I	%	*I*	%	*I*	%	*I*	%	*I*	%	*I*	%
Age (years)												
0	43.2	26.1	86.0	8.7	54.1	11.2	41.8	6.6	148.9	3.6	107.8	5.0
0-5 m	*67.6*	*20.7*	*134.2*	*6.8*	*72.5*	*7.5*	*50.7*	*4.1*	*159.4*	*1.9*	*102.0*	*2.4*
6-11 m	*18.8*	*5.4*	*37.7*	*1.9*	*35.7*	*3.7*	*32.8*	*2.5*	*138.4*	*1.7*	*113.6*	*2.6*
1-4	12.2	28.3	74.8	31.4	33.4	28.4	42.1	25.8	281.2	28.3	149.8	28.5
5-9	10.2	29.7	76.7	39.2	32.8	34.7	47.9	34.1	295.3	36.2	124.9	29.7
10-14	3.2	9.4	22.0	10.6	9.9	9.7	21.1	16.1	110.9	12.8	52.4	11.6
15-19	0.3	1.1	3.6	1.8	1.9	2.0	3.4	2.9	24.8	2.9	15.9	3.6
≥ 20	0.1	5.3	1.4	8.4	1.1	14.1	1.5	14.6	10.8	16.2	7.5	21.5
Total	2.1	100	12.2	100	5.9	100	8.2	100	50.7	100	26.4	100

Seasonal trend of notifications

In March/April 1996, the number of reported cases started to rise (Figure 1). The largest monthly number of cases occurred somewhat later in the year (October 1996) than in the periods 1989 to 1995 and 1997-1998 (mostly August, sometimes July or September).

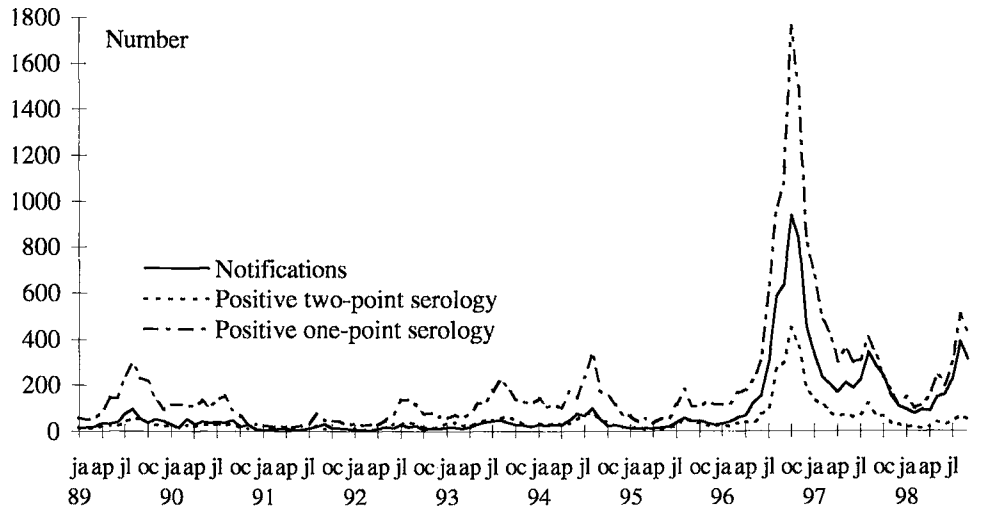

Figure 1. Absolute numbers of pertussis cases per month according to notifications, cases with positive two-point serology, and cases with positive one-point serology

Comparison with positive serology

The seasonal trend for positive two-point serology and positive one-point serology was similar to that of the notification data (Figure 1).

Vaccination status for notifications

The vaccine efficacy estimated by the screening method with notification data was great from 1981 to 1984 (1-4-year-olds: 94% to 99%; 5-9-year-olds: 87% to 100%) and from 1988 to 1993 (1-4-year-olds: 89% to 95%; 5-9-year-olds: 78% to 89%). The estimates were somewhat smaller from 1985 to 1987; they amounted between 72% and 85% for 1-4-year-olds and between 56% and 77% for 5-9-year-olds. The estimates decreased after 1993 (Table 3). They were the smallest in 1996 and could not be estimated in 1997 since the proportion of vaccinated patients exceeded

96%. The vaccine efficacies for almost all strata of diagnosis were greater in 1993 than in the period 1994-1997 (Table 3). There was a decreasing trend, although it was not consistent for all diagnosis strata. The estimates for cases diagnosed microbiologically tended to be the greatest, while the estimates for the cases confirmed by one-point serology tended to be the smallest.

Method of diagnosis for notification from 1993 to 1997

The linkage between the notification database and serodiagnostic database for the period 1993 to 1997 shows the proportion of reported cases confirmed with a microbiological method, with positive two-point serology or epidemiologically, decreased, while the proportion of cases confirmed with positive one-point serology increased (Table 4). The proportion of cases that were confirmed serologically, but that could not be matched with the serological database, also increased. It was not possible to differentiate these cases for positive one-point serology and positive two-point serology. During 1996 and 1997, the proportion of cases confirmed microbiologically by quarter year was rather similar (8.7-10.2%) with the exception of a smaller proportion in the last quarter of 1997 (6.4%). The proportion of cases confirmed by two-point serology ranged between 12% and 19%. In the first two quarters of 1996 (34.9-35.6%) and in the fourth quarter of 1997 (34.3%), the proportion of cases confirmed by positive one-point serology was similar to the proportions of 1994 and 1995. The numbers increased to more than 50% in the fourth quarter of 1996 and the first quarter of 1997. The proportion of cases confirmed serologically that could not be matched with the serodiagnostic database was great(est) in the fourth quarter of 1997 (35.3%).

Estimate of the notification rate

The reported proportion of cases with positive two-point serology increased from 24% in 1993, 26% in 1994, 28% in 1995 to 42-43% in 1996 and 1997. A similar trend was observed for the various age groups.

For positive one-point serology, the reported proportion increased from 6% in 1993, 10% in 1994, 12% in 1995 to 27-29% in 1996 and 1997. The discrepancy was somewhat greater with increasing age. The increase in the reported proportions of cases with positive two-point serology and with positive one-point serology was probably underestimated because the notification database contained serologically confirmed cases that could not be matched with the serodiagnostic database. This proportion was greatest in 1997 (Table 4).

Table 3. Absolute number of reported cases according to vaccination status and estimate of vaccine-efficacy according to method of diagnosis*

		1-4-year-olds		5-9-year-olds	
Year	Method of diagnosis	Number	Vaccine efficacy (in percentages)	Number	Vaccine efficacy (in percentages)
1993	Microbiological	14	93	14	94
	Two-point serology	25	91	28	75
	One-point serology	24	79	26	50
	Other§	35	96	20	90
	Total	98	93	88	84
1994	Microbiological	23	56	15	42
	Two-point serology	36	67	36	83
	One-point serology	63	71	65	#
	Other§	37	89	29	91
	Total	159	77	145	72
1995	Microbiological	14	85	9	67
	Two-point serology	27	82	19	64
	One-point serology	39	64	45	#
	Other§	37	53	27	67
	Total	117	72	100	45
1996	Microbiological	116	67	123	3
	Two-point serology	245	#	288	49
	One-point serology	545	#	782	#
	Other§	290	66	355	40
	Total	1196	34	1548	16
1997	Microbiological	63	51	68	57
	Two-point serology	110	#	131	#
	One-point serology	283	#	345	#
	Other§	244	#	260	#
	Total	700	#	804	#

* A vaccine-coverage of 96% was used to estimate the incidence and vaccine efficacy

§ Epidemiological, serological (differentiation between positive two-point serology and positive one-point serology) not possible, clinical or method of diagnosis unknown

\# Vaccine efficacy could not be estimated as the percentage vaccinated was more than 96%

Table 4. Method of diagnosis for reported pertussis cases from 1993 to 1997

Method of diagnosis*	1993		1994		1995		1996		1997	
	Number	%	Number	%	Number	%	Number	%	Number	%
Microbiological	73	21.1	89	17.1	51	15.0	418	9.9	235	8.8
Two-point serology	100	28.9	116	22.4	62	18.2	760	18.0	383	14.3
One-point serology	87	25.1	180	34.7	119	34.9	2041	48.2	1172	43.9
Serological (not matched)	20	5.8	39	7.5	59	17.3	544	12.9	609	22.8
Epidemiological	38	11.0	40	7.7	27	7.9	178	4.2	96	3.6
Other	28	8.1	55	10.6	23	6.7	290	6.9	176	6.6
Total	346	100	519	100	341	100	4231	100	2671	100

* The method of diagnosis was scored according to the following hierarchy: microbiological, positive two-point serology, positive one-point serology, serological (not matched), epidemiological, and other (clinical, method of diagnosis unknown, negative or inconclusive serology)

Discussion

Interpretations from surveillance data: pertussis trends in the Netherlands from 1976 to 1998

The surveillance data of 1996 show that the unexpected outbreak (as it appeared from notifications) (5) was also observed in hospital admissions, cases with positive serology, and even deaths. The vaccine efficacy estimates that had already declined in 1994 and 1995 declined further in 1996 and 1997. In 1996, according to notifications and serology data, the increase in pertussis incidence among (mostly unvaccinated) children less than 1 year of age was similar to the increase in hospital admissions. However, in older, mostly vaccinated, individuals the increase in hospital admissions was relatively small. Furthermore, we consider it likely that a somewhat smaller epidemic occurred in 1986 and 1987. This is in contrast to what was reported at that time (15,16).

The surveillance data for pertussis in our country were affected by changes in availability of serological tests, interpretations thereof, case definition for notification, and notification rate.
However, we could rely on various surveillance sources, could apply criteria for one-point serology used in recent years to serological data of 1986 and 1987 and were able to match our notification database with our serodiagnostic database. This enabled us to gain a better understanding whether observed changes in surveillance data represented true changes in the underlying incidence of pertussis.

The trend in hospital admissions likely reflects the incidence of severe pertussis. We expect that this surveillance source is less sensitive to changes in the previously described factors. Thus, increasing or decreasing numbers of pertussis cases according to notifications and data of positive serology are likely to reflect (partly) true changes when they are accompanied by similar trends in hospital admissions.

We obtained more insight into the effect of changes in definition of positive serology and case definition for notification, on serological data and the notification data. The current more restrictive criteria for positivity of one-point serology were applied to serodiagnostic data of 1986 and 1987. It was possible to study changes into the notification rate of cases with positive one-point serology and cases with positive two-point serology in the years 1993 to 1997 by linking the notification database with the serodiagnosis.
Furthermore, stratifying notifications according to method of diagnosis enabled us to conclude that the decrease in estimated vaccine efficacy and the shift in 1996 and 1997 towards older age groups in the notifications could only partly be explained by the enhanced application of positive one-point serology.

As a result of great variation in case definitions and in type of laboratory confirmation between countries, figures from notifications are meaningless in the context of international comparisons. For comparison between countries hospital admissions might be more useful, although they are limited to severe pertussis cases only.

Possible factors contributing to the pertussis epidemics

Our results clearly show that pertussis has remained endemic with epidemic peaks in the Netherlands, despite high vaccination coverage. Immunity after infection, as well as after vaccination, is not lifelong. Therefore, waning vaccine-induced immunity has been suggested as an explanation of the re-emergence of pertussis in other countries and probably has contributed to the pertussis epidemic in the 1980s as well as in 1996-1997 (17-19). However, the outbreak in 1986-1987 may also have been associated with the temporary reduction of the potency of the Dutch vaccine from 16 to 10 opacity units per dose in the period 1976 to 1984. Furthermore, the somewhat smaller vaccine efficacy estimates in the years 1986 and 1987 might be explained by greater exposure to *B. pertussis* in epidemic periods than in interepidemic periods (20,21).
As we reported previously, the remarkable increase of reported vaccinated patients over a wide age range, starting 2 years before the outbreak of 1996, suggests a role of a mismatch between circulating and vaccine strains (5,7-9). Antigenic divergence between vaccine strains and clinical isolates was observed for two important protective antigens, pertactin and pertussis toxin (9).

Furthermore, data suggest that the whole-cell vaccine protects better against strains with the vaccine type of pertactin than against strains with nonvaccine types (9).

By analyzing serology data and hospital admission data apart from notification data, we were able to assess the increase in incidence of pertussis in 1996 among (mostly unvaccinated) children less than 1 year of age. The increase in incidence was accompanied by a similar increase in hospital admissions for pertussis in the same age group. This indicates that the virulence of *B. pertussis* for unvaccinated and previously unexposed individuals did not change during the outbreak.
In contrast, for older, mostly vaccinated, individuals, the increase of hospital admissions was smaller than the increase found in other surveillance sources. While the incidence of hospital admissions was greatest for infants less than 1 year old, the incidence in other surveillance sources was greatest among 4-year-olds. Therefore, we postulate that a greater proportion of infected vaccinated individuals expressed clinical symptoms due to the antigenic shifts. This probably leads to a more intense spread of bacteria and thus a greater force of infection in the population. This is shown by the increase of cases in unvaccinated infants.
Despite the findings of antigenic variants of pertactin and pertussis toxin in other countries, no outbreaks similar to those in the Netherlands have yet been observed (22-24). It may be that the vaccines in these countries are potent enough to offset antigenic variation (22). It is also possible that pertussis vaccines protect less well against strains with particular pertactin profiles, which dominate in the Netherlands, but are less common in other countries (22). The Dutch vaccine has been used in the National Immunisation Programme since 1953. No sign of an abrupt deterioration of vaccine quality as determined for product release by the mouse protection test has been found (5). However, the Dutch whole-cell vaccine induces low levels of antibodies against pertussis toxin and filamentous haemagluttinin and high levels of antibodies to agglutinogens and pertactin (6). Perhaps this immunogenicity profile has resulted in a greater vulnerability of the vaccinated Dutch population to antigenic changes in *B. pertussis,* possibly especially with respect to pertactin. We have not yet been able to study a potential effect on the epidemiology of pertussis as a result of a slightly higher content of pertussis toxin in the Dutch vaccine since November 1997.

A re-emergence of pertussis has also been observed in Canada. The similarities to the Dutch re-emergence shed some light on the role of the immunogenicity profile of the vaccine (1,2,5). These similarities are the small proportion of infants affected and large proportion of patients aged 1 to 9 years, estimates of little vaccine efficacy, and lower levels of antibodies against pertussis toxin after vaccination with the Canadian whole-cell vaccine (19,25). This contrasts to the pertussis emergence in the United States, which is accompanied by greater proportions of affected infants and adults and more favorable vaccine efficacy estimates (21,26). The levels of pertussis toxin

antibodies after vaccination were found to be higher for an American whole-cell vaccine, than for the Canadian whole-cell vaccines (25).

Conclusions

We realize that the surveillance data support the hypothesis of the role of antigenic changes in the *B. pertussis* population in the pertussis outbreak, but do not provide a definitive explanation. Furthermore, the indisputable role of whole-cell vaccine in protection against severe pertussis is clearly shown by the sharp decrease in hospital admissions after 12 months of age, as well as by a much smaller pertussis incidence in the past and at present in our country than in unvaccinated populations (27). In such an unvaccinated population, 60% of unvaccinated individuals had experienced clinical pertussis before the age of 10. This is at least 30 times more, even if we assume a notification rate of 25% and an incidence as that observed during the epidemic in 1996. Booster vaccination will be helpful in reducing the pertussis incidence. However, it has to be taken into account that a number of acellular vaccines do not contain the antigenic variants of pertactin and pertussis toxin that dominate in Europe (9,22,23). Furthermore, if pertussis infections are to be postponed until adulthood as a result of boosting, there might be a greater probability of transmission from adults to young, unvaccinated infants. The effects of booster vaccination on the pertussis epidemiology must be monitored carefully, and various surveillance sources must be used to distinghuish surveillance artifacts and real epidemiological effects.

Acknowledgment

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Chapter 9

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

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Submitted

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Abstract

Laboratory confirmation of pertussis by culture, polymerase chain reaction (PCR), or detection of antibody increase in paired sera is hampered by low sensitivity in the later stages of the disease. Therefore, we investigated whether, and at which level, concentrations of IgG antibodies against pertussis toxin (IgG-PT) in a single serum sample are indicative of actual or recent pertussis. IgG-PT, measured by ELISA in U/ml, was analyzed in 7756 population-based sera, in sera of 3491 patients with at least a fourfold increase of IgG-PT, in paired sera of 89 patients with positive cultures and/or PCRs, and in sera of 57 patients with clinically documented pertussis with a median follow-up of 1.4 years. We conclude that, independently of age, IgG-PT levels of at least 100 U/ml are diagnostic of recent or actual infection with *B. pertussis*. Such levels are present in less than 1% of the population and are reached in most pertussis patients within 4 weeks after disease onset and persist only temporarily.

Introduction

Whooping cough is a highly contagious bacterial infection of the respiratory tract, caused by *Bordetella pertussis*. It is most severe in unvaccinated infants. Evidence is increasing that *B. pertussis* infections occur more frequently in older children and adults in vaccinated populations than has been commonly recognized (1-7).These individuals may play an important role in the transmission to infants too young to be vaccinated (5,8-10). Adequate laboratory diagnosis is important for control and prevention of pertussis.

In the Netherlands, the case definition for notification of pertussis includes defined typical clinical symptoms and laboratory confirmation. Laboratory confirmation is defined as either a positive culture or a positive polymerase chain reaction (PCR) for *B. pertussis* or *B. parapertussis*, or positive two-point serology, i.e., a significant increase (at least fourfold) of antibodies against (antigens of) *B. pertussis*. This case definition for notification is highly specific, but it results in low sensitivity, especially when laboratory diagnosis is initiated at a late stage of the disease. Other countries also report that pertussis diagnosis is hampered by low sensitivity (11-15). Culture of *B. pertussis* is laborious and insensitive; the ability to isolate *B. pertussis* by culture decreases progressively during the disease (16,17). The sensitivity of PCR is superior to that of culture; however, this sensitivity, like that of culture, rapidly decreases by the time the paroxysmal phase has developed, and with increasing age (18,19). In the Netherlands, confirmation of suspected pertussis is attempted often by serology. However, in our serodiagnostic practice, for more than 50% of the suspected cases, only one serum sample is submitted or high titers are found in paired sera without a significant increase. Similar problems are also reported by others (20-22).

Because pertussis toxin is expressed only by *B. pertussis* and cross-reacting antigens have not been described (21,23) and because IgG responses occur in most patients with *B. pertussis* infection, we investigated whether, and at which level, titers of IgG-antibodies against pertussis toxin (IgG-PT) in a single serum sample are indicative for actual or recent pertussis.
We analyzed IgG-PT in sera of a large cross-section of the population (N = 7756), in paired sera of patients of all ages in whom clinical suspicion of pertussis was confirmed by at least a fourfold increase of IgG-PT (N = 3491), and in paired sera of patients in whom pertussis had been confirmed by culture of *B. pertussis* or by positive pertussis PCR (N = 89). The course of IgG-PT after natural infection, i.e., the duration of high levels, was assessed in long-term follow-up sera of 57 patients after pertussis had been clinically documented.

Material and methods

Collection of sera and data from population and patients

The cross-section of the general population (N = 7756)

The study design and data collection have been published elsewhere (24). Briefly, eight municipalities with probabilities proportional to their population sizes were sampled within each of five geographical Dutch regions with similar population sizes. An age-stratified sample (classes 0, 1-4, 5-9, ..., 75-79 years) of 380 individuals was randomly selected from each municipality. These individuals were requested to give a blood sample and to fill out a questionnaire in which the participants were asked whether they had had a period with coughing attacks that had lasted for more than two weeks. They were also asked whether a physician had diagnosed pertussis, either during the past year or more than one year previously. The participation rate was 55%. Sufficient serum for pertussis serology was available from 7756 of the 8359 participants. Sera were collected in 1995 and 1996 and stored at -70° C until use.

Patients with serologically confirmed pertussis

Until 1997, the National Institute of Public Health and the Environment was the only laboratory in the Netherlands that performed pertussis serology for patients with a suspected pertussis infection. Routinely, the submitted sera were assayed for both IgG-PT and IgA against *B. pertussis*. In all cases, if the date of onset of symptoms and/or date of sampling of serum was missing upon submission of serum, a standard questionnaire was sent to collect the data. From January 1989 onwards, all data and results were registered in an electronic database.
For the purpose of this study, patient data and serologic results obtained in the period 1989-1996 from 3491 patients in whom the clinical suspicion of pertussis had been confirmed by the detection, in paired sera, of ≥ fourfold increase of IgG-PT to ≥20 U/ml.
Likewise, data were analyzed for 15319 patients whose first submitted serum sample contained ≥100 U/ml IgG-PT without (in case of paired sera) a fourfold IgG-PT increase.

Patients with typically symptomatic infection with B. pertussis and their longitudinal sera

During the period 1989-1998, we obtained follow-up serum samples from 57 patients with a clinical diagnosis of typical pertussis (paroxysmal cough lasting more than two weeks) so that we could study the longitudinal course of IgG-PT after infection.
Twenty-three patients showed at least a fourfold increase in IgG-PT in paired sera, and 34 patients had an IgG-PT in a first serum sample of at least 75 U/ml. The IgG-PT level was at least

100 U/ml for 31 of these 34 patients. The follow-up period varied from 6.5 months to 6.7 years after the acute phase of infection (mean: 1.8 years; median: 1.4 years). The number of serum samples collected in the follow-up periods varied from two to seven (mean and median: three). All patients were from a single pediatric practice. Only those patients participated who, continued to be treated by the pediatrician after the episode of pertussis because of other medical conditions (mostly allergic conditions and/or asthma) or who consulted the pediatrician again at a later stage because of new medical problems. In addition, sera from parents with clinical pertussis were selected. The median age of the patients in which the longitudinal course of IgG-PT was studied was 3.5 years (range: 0-35 years). Ten patients were less than 6 months old; 7 patients, 6-11 months old; 19 patients, between 1 and 4 years old, 16 patients, between 5 and 9 years old; 2 patients, 11 and 12 years old; and 3 patients, 30 to 35 years old. Thirty-nine of the patients were vaccinated, 6 patients were unvaccinated, and for 12 patients the vaccination status was unknown. In all cases the follow-up sera used for the study were "left over" from samples obtained for some other diagnostic procedure. Informed consent for the study was obtained from the patients or their parents.

Patients with PCR and/or culture-proven pertussis

In the period 1993-1998, the diagnosis of pertussis for 89 patients had been confirmed by culture of *B. pertussis* and/or a positive pertussis-specific PCR. Paired sera from these patients had been submitted for serology. Fifty-eight of the 89 patients had participated in a clinical study to assess the sensitivity of the pertussis/parapertussis PCR in comparison with culture and serology (18). For each of the remaining patients, a pertussis PCR test was performed in the regional public health laboratory in Tilburg, the Netherlands. In all cases, the first serum of the pair had been obtained on the same day that material for culture and/or PCR had been obtained; the second sample had been obtained 2 to 4 weeks later. Of the 89 patients, 37.1% were 0-5 months old; 7.9%, 6-11 months; 21.3%, 1-4 years; 25.2%, 5-9 years; 2.2%, 10-14 years, and 5.6%, $\geq$ 15 years.

In house IgG-PT ELISA

The patient sera had been submitted immediately after sampling and were assayed in the routine setting of the serology laboratory of our institute within 4 days after receipt. The population sera, which had been collected in 1995/1996, were assayed in 1997/1998 in the same routine setting at a rate of approximately 200 sera per week. The IgG-PT was measured by ELISA as previously described (18). In short, the procedure was as follows: purified PT (source: National Institute of Public Health and the Environment) was coated to 96-well ELISA plates after precoating with fetuin (50 mg/l in phosphate-buffered saline). Peroxidase labeled rabbit antihuman IgG was used

as a conjugate, and 3,3',5,5' tetramethylbenzidine (TMB) was used as the substrate. A negative, low-positive, and medium-positive control serum with defined IgG-PT content was run on each plate. The sera were tested in duplicate in a 1:100 and 1:400 dilution against serial dilutions of a positive reference serum with a range of 1.6-100 "local" U/ml. The optical density (OD) of the 1:100 dilution was used for calculation of the IgG-PT concentration. When the OD of the 1:100 dilution of a serum was above the range that constituted the steep part of the dose response curve, the OD of the 1:400 dilution was used.
Due to the use of only two dilutions for the sera, the IgG-PT assay has an upper limit (500 U/ml) above which the values are not further differentiated. The lower detection limit of the assay is 5 U/ml.
Results are expressed in "local U/ml". The reference serum was also calibrated against the FDA preparation Lot 3 (FDA, Laboratory of Pertussis, Rockville, MD). The formula for conversion of "local" to "FDA" U/ml appeared to be: local U/ml x 0.8 = FDA U/ml within this assay.

Data analysis

For the cross-sectional population-based study, frequencies of IgG-PT levels from <5 U/ml (lower detection limit) to ≥500 U/ml (upper differentiation limit) within each municipality were weighted by the proportion of the age group in the population. To produce national estimates for the 1, 2.5, 10, 25, 50, 75, 97.5, and 99 percentiles, these weighted and age-specific frequencies were averaged over the 40 municipalities (25).
The following age groups were separately analyzed: 0-5 months, 6-11 months, 12-17 months, 18-23 months, 2 years, 3 years, 4 years, 5 to 9 years, 10 to 14 years, and ≥15 years of age.
The proportions with IgG-PT <5U/ml, 5-9 U/ml, 10-49 U/ml, 50-99 U/ml, 100-499 U/ml, and ≥500 U/ml were also assessed. The proportion of these groups that reported a pertussis diagnosis or a period with coughing attacks during the past year was calculated. The proportion of those who had a pertussis diagnosis and/or coughing attacks more than one year ago and the proportion without any pertussis diagnosis or coughing attacks were also calculated.
Likewise, for the patients with serologically proven pertussis (i.e., ≥ fourfold increase of IgG-PT in paired sera), the total and (similar) age-specific 1, 2.5, 10, 25, 50, 75, 97.5, and 99 percentiles for IgG-PT levels were calculated in the second sera of the serum pairs. The median duration of disease at the time of initiation of laboratory diagnosis, i.e., the time of sampling of first sera, was assessed for these patients in the age groups 0-5 months, 6-11 months, 1 year, 2 years, 3 years, 4 years, 5 to 9 years, 10 to 14 years, and ≥15 years of age.
The median duration of the disease was also assessed for the same age groups for the selection of patients from the serological database with ≥100 U per ml at the time of the first serum sampling without a fourfold IgG-PT increase.

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

To study the longitudinal course of IgG-PT levels after pertussis infection for patients in the follow-up study, the association between the IgG-PT level and time in ^{2}log days after the first day of illness (and the effect of age) was analyzed with a mixed linear model (PROC MIXED in SAS version 6.12) (26). A ^{2}log transformation of both the IgG-PT level in U per ml and of time in days since the first day of illness was performed. This way, linear regression yielded the best fit.
For those patients with follow-up data 0-5 months, 6-11 months, 12-23 months, 2-3 years, and 4-7 years after onset of the disease, the proportions with IgG-PT levels <20 U/ml, 20-49 U/ml, 50-99 U/ml, and ≥100 U/ml were calculated. If multiple serum samples were available for one patient within one follow-up period, the serum sample with the highest IgG-PT level was used.

Results

The IgG-PT distribution in the population compared to the IgG-PT distribution in second sera of serum pairs of patients with serologically confirmed pertussis: choice of cut-offs to be validated

The IgG-PT levels in individuals of the population-based study were low in comparison to those in second sera (i.e., reconvalescence sera) of patients with serologically confirmed pertussis; these distributions showed little overlap (Figure 1).
In the population-based study, the median IgG-PT was 6 U/ml, and the 97.5 and 99 percentile, 69 U/ml and 97 U/ml, respectively. In the second sera of patients with serologically confirmed pertussis, the median IgG-PT was ≥500 U/ml; the 10 percentile was 66 U/ml (Figure 1).
In the population-based study, the median IgG-PT was <5 U/ml in the age group <10 years old, rose to 5 U/ml for 10 to 14-years-olds, and was 7 U/ml for 15 years old or older (Figure 1). For those 0-5 months, 6-11 months, and 18-23 months old, the 97.5 percentile was below 20 U/ml, while in the other age groups the values given by the 97.5 percentile ranged from 26 U/ml (4-year-olds) to 98 U/ml (10 to 14-year-olds). Further differentiation in 5-year age classes from 15-19 years to 75-79 years showed a stable IgG-PT distribution for those aged ≥15 years (data not shown separately). With the exception of the 10 to 14-year-olds, the age-specific 99 percentile was below 100 U/ml.
The percentages with undetectable IgG-PT (i.e., <5 U/ml) decreased from 85.5% for infants aged 6-11 months to 67.4% for infants 12-17 months and increased again to 79.4% at the age of 4 years (Figure 1). The proportion with undetectable IgG-PT decreased to 31.7% in those ≥15 years of age. From this age onwards, this percentage remained stable (not shown separately).

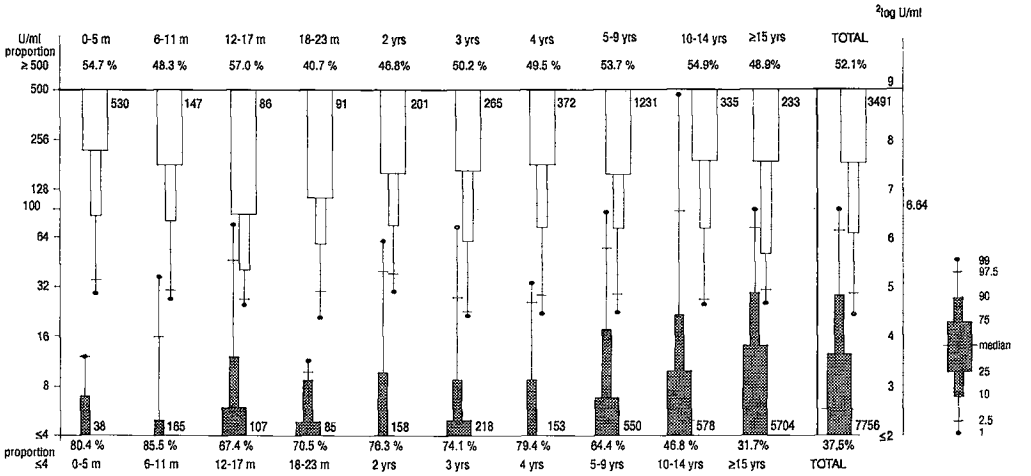

Figure 1. IgG-PT distribution in the population-based study and in (second sera of) pertussis patients with at least a fourfold increase in IgG-PT level in paired sera.

(Results for the population-based study are given in gray bars and results for second sera of pertussis patients are given in white bars; total numbers of patients are given aside the bars)

The median and 10 percentile of the distributions of IgG-PT in second sera of patients with serologically proven pertussis showed little variation in the different age groups; median IgG-PT ranged from 316 U/ml to ≥500 U/ml, and the 10 percentile ranged from 40 U/ml to 87 U/ml (Figure 1). The percentage of patients with an IgG-PT level of ≥500 U/ml ranged from 40.7% to 57.0% within the various age groups (Figure 1).

Thus, the IgG-PT distributions in the population differed for the various age categories, but IgG-PT immune responses in pertussis patients did not. Therefore, for further assessment of specificity and sensitivity of certain IgG-PT levels for recent/actual infection with *B. pertussis,* we chose cut-offs of IgG-PT levels which were age independent, i.e., based on the comparison of the IgG-PT distribution in the total population and the IgG-PT distribution in second sera of the total group of serologically confirmed pertussis patients. Cut-offs of 50 U/ml and 100 U/ml were chosen because IgG-PT levels ≥50 and ≥100 U/ml were detected in respectively 92.7% and 81.0% of (second sera of) patients with serologically confirmed pertussis. Such levels were rare: respectively, 3.6% and 0.8% in the general population. That is to say, they gave specificities of such values as 96.4% and 99.2%, respectively. The specificity of the proposed cut-off of ≥100 U/ml was maximally 100% for the ages of 0-5 months, 12-17 months, 18-23 months, and 2 years old; minimally 97.9% for 10 to 14-year-olds; and for the cut-off, ≥50 U/ml, maximally 100% for the ages of 0-5 months and 18-23 months and minimally 96.2% for 10 to 14-year-olds and ≥ 15-years-olds.

Validation of the proposed IgG-PT cut-offs as markers for recent or actual infection with *B. pertussis*

The relation between high IgG-PT levels in the population and recall of pertussis diagnosis and/or paroxysmal coughing

In the population-based study, while 9.6% of those with an IgG-PT level of <5 U/ml reported coughing attacks or pertussis during the last year, this percentage was statistically significantly greater: 19.9% for those with an IgG-PT level between 50 and 99 U/ml, and 25.5% for those with an IgG-PT level ≥100 U/ml (Table 1).
The percentage of individuals with a diagnosis of pertussis or who had coughing attacks more than one year ago showed a smaller increase with increasing IgG-PT. The differences in the percentages were not statistically significant (Table 1).

The longitudinal course of IgG-PT levels after infection

In each of the 57 patients with clinical pertussis for whom there were follow-up serum samples, the IgG-PT decreased with time after the onset of the disease (Figure 2). In the mixed linear model with the IgG-PT in ^{2}log U/ml and time from the onset of the disease in ^{2}log days, the intercept amounted to 14.61 and the slope was -1.128. Thus, this model predicts that the mean time of persistence of an IgG-PT level ≥100 U/ml amounts to 134 days (4.4 months) and that after 365 days, a level of 32 U/ml is reached. Although IgG-PT levels of ≥500 U/ml were not further differentiated, the slopes did not change statistically significantly assuming that the levels of ≥500 U/ml were 1000 U/ml or restricting the analysis to those samples with IgG-PT levels <500 U/ml. No effect of age or vaccination status was shown in the mixed linear model.
The data points for each individual patient were connected linearly. The resulting lines show that the IgG-PT level were below 100 U/ml within 1 year for 47 of the 57 patients. For 7 of the remaining 10 patients, the IgG-PT levels decreased below 100 U/ml during the subsequent follow-up period, which ranged from 1.4 years to 4 years. For 2 of the remaining 3 patients, the follow-up period was less than 1 year. For these 2 patients, the IgG-PT levels amounted to 160 U/ml and 304 U/ml after 0.84 and 0.92 years of follow-up, respectively. The remaining patient had an IgG-PT level of 252 U/ml 1.1 year after the first day of illness.

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

IgG	N	<5 IU/ml	(95% CI)	5-9 IU/ml	(95% CI)	10-49 IU/ml	(95% CI)	50-99 IU/ml	(95% CI)	≥100 IU/ml	(95% CI)
Pertussis diagnosed and/or coughing attacks during last year*	886	9.6	(8.0-11.3)	11.6	(9.4-13.8)	11.3	(9.7-12.9)	19.9	(13.0-26.9)	25.5	(12.2-38.8)
Pertussis diagnosed and/or coughing attacks more than one year ago; No pertussis diagnosed and no coughing attacks during last year†	463	5.5	(4.3-6.7)	6.1	(4.6-7.6)	6.7	(5.2-8.5)	7.3	(3.5-11.0)	14.1	(2.7-25.5)
No pertussis or coughing attacks	6407	84.9	(82.8-87.0)	82.3	(79.9-84.6)	81.8	(80.1-83.5)	72.8	(65.2-80.5)	60.4	(45.3-75.5)
Total	7756	100%		100%		100%		100%		100%	

* Among the 886 individuals, 15 individuals reported pertussis and 12 of these 15 individuals reported coughing attacks.

† Among the 473 individuals, 126 individuals reported pertussis and 45 of these 126 individuals reported coughing attacks.

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

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

	0-5 months	6-11 months	12-23 months	2-3 years	4-7 years
Number	57	30	25	14	8
≥ 100 U/ml	90%	20%	16%	7%	0%
50-99 U/ml	11%	40%	12%	7%	0%
20-49 U/ml	0%	13%	28%	36%	13%
< 20 U/ml	0%	27%	44%	50%	88%

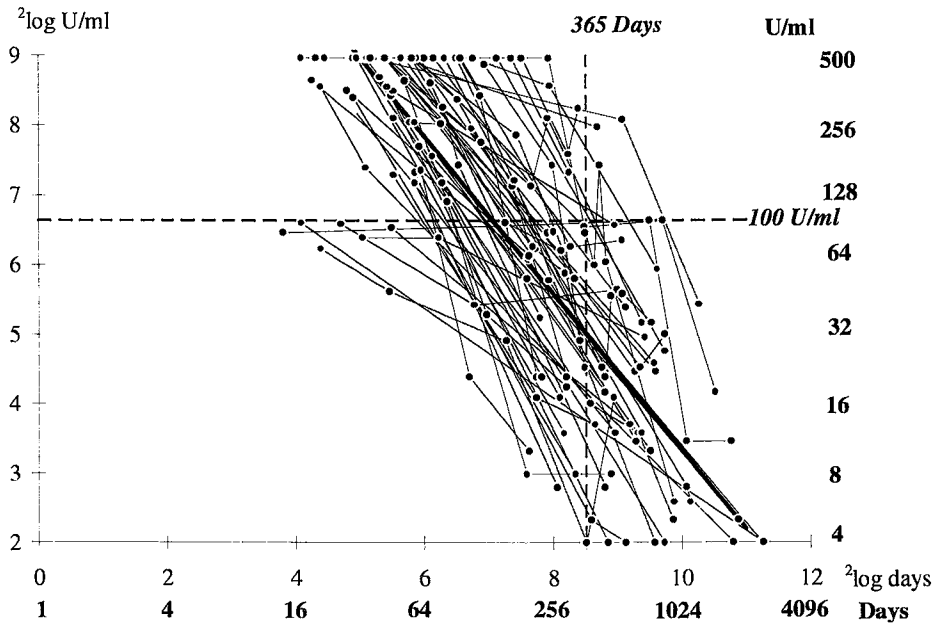

Figure 2. IgG-PT levels (in ^{2}log U/ml) versus time elapsed (in ^{2}log days) since date of onset for 57 patients with clinical pertussis

Time between onset of the disease and development of high IgG-PT levels

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

The results given in table 3 also show the number of patients with an IgG-PT level ≥100 U/ml in a first serum that in our serodiagnostic database of 1989-1996 is considerably larger (4.5-fold) than the number of patients with at least a fourfold increase in IgG-PT. This discrepancy increases with age from onefold for 0 to 5-months-olds to 13.6-fold for ≥15 years of age.

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

	Patients with at least a four-fold IgG-PT rise				Patients with at least 100 U/ml in first serum sample without a four-fold IgG-PT rise			
	Number	Median (days)	2.5 - 97.5 percentile	Mean (days)	Number	Median (days)	2.5 - 97.5 percentile	Mean (days)
0-5 months	517	14	(2-53)	17.0	497	30	(4-108)	36.8
6-11 months	144	14	(2-61)	16.8	305	29	(2-105)	35.4
1 years	174	15	(2-62)	19.2	426	28	(4-122)	36.3
2 years	195	15	(4-88)	19.6	763	30	(6-109)	38.1
3 years	262	18	(3-60)	19.7	966	30	(7-119)	38.5
4 years	362	21	(5-55)	21.9	1499	31	(7-112)	39.0
5-9 years	1191	18	(3-58)	20.6	5655	31	(7-103)	37.6
10-14 years	327	18	(4-61)	21.3	2180	31	(7-111)	40.0
≥15 years	222	16	(3-67)	19.3	3028	30	(6-108)	37.2
Total	3394*	17	(3-59)	19.8	15319	30	(6-109)	38.0

* N < 3491 due to missing values for time of disease onset

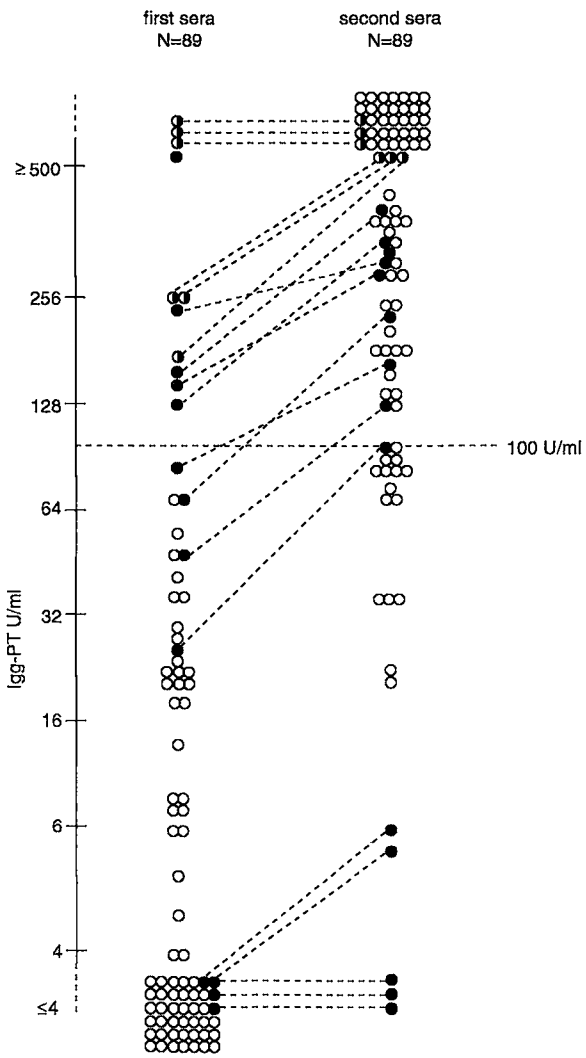

Figure 3. IgG-PT distribution in the first and second serum samples of patients with a positive culture and/or PCR for Bordetella pertussis

White rounds indicate sera with at least a fourfold increase in IgG-PT to a level of at least 20 U/ml; Black rounds indicate sera without fourfold increase in IgG-PT; pairs of sera are connected with a dotted line; Half black, half white rounds indicate sera in which the degree of increase of IgG-PT could not be determined due to the upper limit of differentiation of the IgG-PT assay of ≥500 U/ml; pairs of sera are connected with a dotted line.

Sensitivity of the proposed cut-offs for IgG-PT in PCR and/or culture-confirmed pertussis cases

Among the 89 patients for whom pertussis was confirmed by a positive culture and/or a PCR both in the IgG-PT distributions in the first and second serum samples, no statistically significant differences were found between age groups.
The distributions of IgG-PT in first and second sera of the serum pairs of these patients are shown in Figure 3. In 3 of the 89 patients, IgG-PT was undetectable (<5 U/ml) in both the first and the second serum samples. In two other patients the IgG-PT rose from <5 U/ml to 7 and 8 U/ml. In 69 patients a ≥ fourfold increase of IgG-PT was detected, in 11 patients the IgG-PT in the first serum sample was ≥ 100 U/ml and no fourfold rise was detected, and in 4 patients the rise in IgG-PT was less than fourfold and the IgG-PT in the first serum sample less than 100 U/ml. Thus, if detection of a fourfold increase or more of IgG-PT in paired sera and detection of IgG-PT ≥100 U/ml in a single serum sample are used as criteria for serodiagnosis of actual/recent infection with *B.pertussis*, the sensitivity of IgG-PT serology in this golden standard group of patients is enhanced from 77.5% (69/89) to 89.9% (80/89). Overall, the sensitivity of an IgG-PT level of ≥50 and ≥100 U/ml amounted to 88.8% and 76.4%, respectively: i.e., 79 and 68 of the 89 patients had an IgG-PT level of ≥50 U/ml and of ≥100 U/ml in first and/or second serum samples.

Discussion

Our results show that an IgG-PT level of at least 100 U/ml is a specific tool in laboratory confirmation of patients with a suspected pertussis infection in the Netherlands. The levels of IgG-PT in the Dutch population are lower than, and overlap only slightly, IgG-PT levels that are reached in patients with clinical symptoms of pertussis and a significant immune response to *B. pertussis*. IgG-PT levels of least 100 U/ml were observed in less than 1% of the overall general population, varying between maximally 2.5% for 10 to 14-year-olds and less than 0.5% for those 2 years of age or less. Furthermore, such levels were present in the second serum sample of 80% of those patients who had at least a fourfold increase in IgG-PT.

Our longitudinal study of pertussis patients shows that the levels decreased within less than 1 year to a level below 100 U/ml after natural infection with *B. pertussis* for almost all patients who had had high IgG-PT levels. The regression model predicts that a level of 100 U/ml is reached in 4.5 months; and a level of less than 40 U/ml, 1 year after onset of the disease. For 7 of 8 patients with a longer follow-up time, the IgG-PT levels were below 20 U/ml 4-7 years after the disease onset. Thus, IgG-PT levels of at least 100 U/ml for patients with suspected whooping cough are indicative of *recent* or *actual* infection with *B. pertussis*.
These results are also important for the interpretation of the serological profile of IgG-PT in the general population to assess the infection rate of *B. pertussis*. Cumulative incidence cannot be

calculated directly from the seroprofile as others have done (27). The decline of IgG-PT after infection with *B. pertussis* has to be taken into account.

In our population-based study, the percentage of individuals who reported pertussis or coughing attacks in the last year increased from 10% for those with IgG-PT levels less than 5 U/ml to 26% for those with at least 100 U/ml. This finding offers further support for our conclusion that a high IgG-PT level is indicative of recent or actual infection with *B. pertussis*.
Ten percent of the individuals in the population study with an IgG-PT level less than 5 U/ml reported pertussis or coughing attacks in the last year. On the one hand, this may be explained by other respiratory tract infections that cause "pertussis-like" symptoms (28), a rapid decline of previously high IgG-PT level or the absence of IgG-PT response after *B. pertussis* infection. It is also possible that not all subjects answered the question properly. On the other hand, the large percentage (60%) of individuals with high antibody titers who did not report pertussis or long-lasting coughing attacks might be due to very mild, atypical, or even asymptomatic infection with *B. pertussis*. In a household exposure study, a *B. pertussis* infection was shown to exist in 46% of the exposed subjects who remained well (10). In another study, only 26% of the adults with laboratory evidence of a *B. pertussis* infection reported recent symptoms compatible with pertussis (14).

Among those with an IgG-PT level of at least 100 U/ml, the percentage of individuals who reported coughing attacks or pertussis more than 1 year previously was higher (14%), but not statistically significant, when compared to those with an IgG-PT level of less than 5 U/ml (6%). This supports our finding that IgG-PT decreases after *B. pertussis* infection. As our population-based serum bank was not established specifically to study pertussis, but to study vaccine-preventable diseases (a broader objective), the exact time of illness was not further specified (24). Therefore, we could not study more accurately the relation between the level of IgG-PT to the time elapsed since the coughing attacks or pertussis. Theoretically, a patient with suspected pertussis may suffer from another disease that causes similar symptoms at the moment of blood sampling and may have had a *B. pertussis* infection a few months earlier.

Although IgG-PT is induced after whole-cell vaccination only in children aged 12-17 months, and thus after the fourth dose given at the age of 11 months, a temporary and small increase was observed. These results are consistent with observations in a vaccine trial showing very low levels of IgG-PT after the first to third vaccinations and a small increase just after the fourth vaccination, as Nagel observed (29,30). Thus, it is very unlikely that high levels of IgG-PT are induced by previous vaccination with Dutch whole-cell vaccine. However, other vaccines might induce higher IgG-PT levels as the response to pertussis toxin varies between different whole-cell vaccines and acellular vaccines (31-34). However, even when a level of at least 100 U/ml is reached, it is likely

to decline shortly afterwards (32-34). Giuliano et al. (32) show that mean titers were close to the limit of detection 15 months after the primary immunization with acellular vaccine. High IgG-PT levels must be interpreted more cautiously in children recently vaccinated with a vaccine known to induce relatively high levels of such antibodies.

In addition to specificity, both sensitivity and positive predictive value are important for diagnostic tools. Using paired sera of patients with positive PCRs and/or positive cultures (golden standard group), a sensitivity of IgG-PT of at least 100 U/ml was 76%. At least a fourfold increase was found in most of the remaining patients in whose paired sera the IgG-PT level remained below 100 U/ml. One might speculate that a level of at least 100 U/ml may have been reached at a later point. Only 6% of all patients had very low IgG-PT levels in both sera without significant dynamics.
With the exception of those aged 10-14 years, a level of IgG-PT of at least 100 U/ml exceeded the 99 percentile in the general population. Furthermore - as already described - it was likely that individuals in the general population with IgG-PT levels above this value had had a recent or actual *B. pertussis* infection. Based on this 99 percentile, the positive predictive value will still amount to 90% and 80%, assuming the proportions of true pertussis patients to be 9% and 4%, respectively among those who submitted serum samples (i.e., 9 / (9 +1) and 4 / (4 + 1)). However, depending on the clinical presentation and the epidemiological situation, the a priori chance of true positivity in most cases will be higher. Using a more conservative estimate, i.e., a 97.5 percentile in the general population, the positive predictive value will not be below 80%, assuming a percentage of true positives of 10%.
Even an IgG-PT level of at least 50 U/ml has some predictive value, as it amounts to 70%, assuming a percentage of true positives of 10%. This suggests that the diagnosis of pertussis is likely among patients with clinical symptoms of pertussis with such IgG-PT levels. However, we interpret such a result as indicative of, but not definite proof of a recent *B. pertussis* infection and we advise submission of a second serum sample. If no further change of IgG-PT level has occurred as evidenced by a second serum sample, we conclude that "recent or actual infection with *B. pertussis* is possible".

Serological data at our laboratory show that using our cut-off value for the IgG-PT level of at least 100 U/ml would increase the number of patients with serologically proven pertussis by more than fourfold. The increase is smallest for infants and greatest for adults, which is probably related to a longer delay in consulting a physician and/or initiating laboratory testing in older children and adults. This is supported by the similar median time (28-30 days) between the first blood sampling and the onset of symptoms for the various age groups for those with an IgG-PT level of at least 100 U/ml.

For patients with at least a fourfold increase in IgG-PT the median time (17 days) between the first blood sampling and the first symptoms is about 2 weeks shorter than that of those without a fourfold increase and IgG-PT levels above 100 U/ml. The most useful method for pertussis diagnosis depends on the time of initiation. PCRs and cultures are most useful early in the disease. However, if they are negative, the diagnosis is indeterminate, and serology tests should be initiated. Late in the disease, PCRs and cultures are fairly insensitive (with an exception for infants less than 1 year old) and serology is then the first method of choice (12,18,19).

To diagnose pertussis other investigators have also used single serum samples from a control group for defining a cut-off, but most studies were limited to a specific study setting and were not meant for routine diagnosis (3,4,6,7,9,11,12,15). Our control group consisted of a large number of participants from a population-based study, so that the representativeness is probably better guaranteed.

We do not share the opinion of Cattaneo et al. (35) that including a few individuals with a recent *B. pertussis* infection in the control population refutes the use of this population as a reference population. This might lead merely to a somewhat less sensitive cut-off value, as the 99 percentile might be slightly overestimated.

Cattaneo et al. point out that it is unlikely that a single serology value can be used to define infected persons in a broad age range because age, geographic area, prevalence of infection, and history of vaccination all have to be taken into account (35). Yet, an IgG-PT level of at least 100 U/ml in a single serum sample might be a specific diagnostic tool for pertussis in other countries too. After all, it is likely that such high IgG-PT levels will not or will be reached only temporarily no matter which vaccine is used. After the initial increase, the IgG-PT level decreases again after *B. pertussis* infection, high predictive values are calculated under different assumptions on prevalence of infection, and finally, a large proportion of individuals with a *B. pertussis* infection show high IgG-PT levels later in the disease. Thus, we believe that high IgG-PT levels could provide a useful laboratory tool for diagnosis of pertussis in both the individual patient and epidemiological studies (12,15,20). It might be worthwhile to validate our results in other countries.

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Chapter 10

General discussion

Results from a nationwide, population-based, cross-sectional study on diphtheria, tetanus and poliomyelitis and results from studies on surveillance and seroepidemiology of pertussis are described in this thesis.
The age-specific prevalence of antibodies has given insight into diphtheria, tetanus and poliomyelitis immunity in the general population and in differences in immunity between the general population and orthodox reformed groups refusing vaccination in the Netherlands. The studies on pertussis surveillance have provided information on the occurrence of pertussis in the Netherlands and on possible factors involved in its re-emergence. Finally, seroepidemiological studies on pertussis have given insight into pertussis diagnosis based on high levels of IgG antibodies against pertussis toxin in a single serum sample.
The main findings of the studies are summarised in the first part of this chapter. Then some methodological considerations and the implications of the findings for public health and future research are described.

Main findings

Part 1. A population-based serum bank

Implementation of the population-based design for collecting sera for public health research with the primary focus on vaccine-preventable diseases (Chapter 2) resulted in an overall response rate of 55.0% for serum collection and questionnaire data in the nationwide sample (N = 8359) and of 52.5% in the low vaccine coverage sample (N = 1589). As a result of this parallel study in municipalities with low vaccine coverage, we had access to about 240 members of orthodox reformed groups who refuse vaccination.
The nonparticipation study (Chapter 3) shows that various characteristics of individuals were independently associated with (non)participation. Various groups of (non)participants were distinguished. There were initial and additional participants, nonparticipants who completed the (nonresponse) questionnaire and absolute nonparticipants for whom only data from the municipal registry were available. Some variables affected each form of (non)participation similarly; other variables affected each form differently. For example, for some variables the difference between participants and nonparticipants was slightly smaller when additional participants were included, while the difference was slightly larger for other variables. Exclusion of absolute nonparticipants, i.e. restricting nonparticipants to those who answered a questionnaire, also affected the association with nonparticipation considerably for some variables.

Part 2. Immunosurveillance for diphtheria, tetanus and poliomyelitis

The results for the studies on seroprevalence of diphtheria, tetanus and poliomyelitis are given in Chapters 4 to 6. Specific antibodies to these diseases give insight into the immunity of the population, since they correlate with protection.
The age-specific prevalence of diphtheria antitoxin antibodies (≥ 0.01 IU/ml), of tetanus antitoxin antibodies (≥ 0.01 IU/ml) and of neutralising antibodies against poliovirus types 1 and 2 (≥ 1:8) in our nationwide sample was more than 92.5% for individuals aged less than 45 years. These individuals were born after the introduction of mass vaccination. The prevalence of neutralising antibodies for poliovirus type 3 was more than 90% for those younger than 30 years and more than 80% for 30 to 44-year-olds. For older individuals, namely, those born before the introduction of vaccination, a sharp decrease with increasing age was observed in the prevalence of antitoxin antibodies against diphtheria and tetanus; for individuals aged 70 to 79 years it amounted to 50% or less. The prevalence of neutralising antibodies against poliovirus types 1, 2 and 3 remained at 80% or more for older age groups.

Among orthodox reformed individuals often refusing vaccination, the prevalence of antibodies was less than that among individuals from the nationwide sample, both for those younger than 45 years and those aged 45 years and more. It amounted to 40% or less for diphtheria antitoxin antibodies and tetanus antitoxin antibodies, while it was 70% or less for neutralising antibodies against poliovirus types 1, 2 and 3. The seroprofiles showed an effect of the recent poliomyelitis outbreaks in 1978 and in the period 1992-1993 among orthodox reformed groups, but not among other participants.

For individuals aged 10 to 34 years who were completely vaccinated according to the National Immunisation Programme standards (i.e. at the ages of 3, 4, 5, 11 months, 4 and 9 years), the geometric mean titres for diphtheria, tetanus and poliomyelitis decreased with increasing age. However, the prevalence of antibodies in this subgroup was at least 98% for diphtheria, tetanus and poliovirus types 1 and 2, and at least 94% for poliovirus type 3. The decline in geometric mean titres with age was similar for diphtheria antitoxin antibodies and tetanus antitoxin antibodies. However, since the geometric mean titres were higher for tetanus than for diphtheria for recently completely vaccinated 10 to 14-year-olds, the tetanus antitoxin antibody levels were higher for 30 to 34-year-olds than the levels for diphtheria. For all three diseases, the geometric mean titres for individuals aged 20-34 years with evidence of revaccination were similar to those for 10 to 14-year-olds who were completely vaccinated, but without evidence of revaccination.

Part 3. Surveillance and seroepidemiology of pertussis

Surveillance data for pertussis are presented in Chapters 7 and 8. The number of pertussis patients increased sharply in 1996 and 1997 as compared with the number in the period 1989 to 1995 according to notifications, serology data and hospital admission data. The increase was largest for notifications and smallest for hospital admissions. For children less than 1 year old, the increase in notifications and positive serology was similar to the increase in hospital admissions. In older individuals, the increase in hospital admissions was smaller than the increase from other surveillance sources. From 1994 onwards, the vaccine efficacy estimated from notification data was less than that from 1989 to 1993. These estimates were lowest in 1996 and 1997. The proportion of reported cases with positive two-point serology increased from 24% in 1993 to 40% in 1996 and 1997. For cases with positive one-point serology, this proportion increased from 6% in 1993 to 29% in 1997.

Finally, Chapter 9 describes the study in which we investigated whether, and at which level, concentrations of IgG antibodies against pertussis toxin (IgG-PT) in a single serum sample are indicative of actual or recent *Bordetella pertussis* infection. IgG-PT is specific for *B. pertussis* and responses occur in most patients with a *B. pertussis* infection. IgG-PT levels of at least 100 U/ml were present in less than 1% of the participants in the population-based study, which means a specificity of 99%. Such levels were present in more than 80% of second sera of patients with serologically proven pertussis, i.e. with at least a fourfold increase in IgG-PT in paired sera. Follow-up of patients with clinical pertussis showed that the IgG-PT level was less than 100 U/ml in more than 80% of the patients less than 1 year after the onset of symptoms. For patients with positive pertussis polymerase chain reaction or culture, the sensitivity of a IgG-PT level of at least 100 U/ml amounted to 76%. The number of patients with IgG-PT levels in a first serum sample of at least 100 U/ml in our serodiagnostic database was more than fourfold greater than the number of patients with positive two-point serology. The increase was smallest for infants less than 6 months of age and largest for individuals aged 15 years or older.

Methodological considerations

There are several methodological issues that should be considered in interpreting the results of the studies described. For the population-based studies on diphtheria, tetanus and poliomyelitis, we discuss the possible bias due to selective participation, the age-cohort effect in association with the persistence of antibodies after vaccination and the validity of vaccination status. The validity of pertussis surveillance data and of estimations of vaccine efficacy from these data is discussed afterwards. Finally, some issues about interpreting the results from the study on high IgG-PT levels as a diagnostic tool for pertussis are discussed.

A population-based serum bank and immunosurveillance of diphtheria, tetanus and poliomyelitis

Selective participation

Inevitably, many serosurveys are being performed on residual sera, i.e. sera from blood donors or sera taken for clinical reasons. It is dubious to what extent sera from these sources can be used to make valid population estimates. Blood donors might be more health conscious, for example, while patients who have a blood sample taken for clinical reasons differ from the general population in that they are ill somehow. To overcome this problem and to be able to collect additional information about participants, we used a population-based approach.
Since participants had little personal interest in participating, and since many people dislike blood sampling, the participation rate of 55% was satisfactory. However, despite an appropriate sampling frame, the percentage of nonparticipants implies that our serum collection may not be representative of the general population. Information available about all participants and all nonparticipants shows that some variables, such as age and sex, were indeed associated with nonparticipation (Chapter 3). However, the effect of differential probabilities of response for these variables on the population estimate amounted to less than one standard error for diphtheria, tetanus and poliomyelitis (Chapters 4, 5 and 6). They were therefore ignored. The absence of an effect of nonparticipation on these estimates was mainly the result of correcting for differential response (age), lack of association with prevalence of antibodies (marital status, region) or being a small subgroup (nationality).

The interpretation of the association of other variables with nonparticipation in the analysis restricted to those nonparticipants who filled in a questionnaire was more difficult. As described in Chapter 3, this might lead to biased insight into nonparticipation selection, since those nonparticipants who filled in a questionnaire might not be representative of all nonparticipants. The most important determinant on which no information was available for all (non)participants was vaccination status.

Perhaps our nationwide estimates of prevalence of diphtheria, tetanus and poliomyelitis antibodies were slightly overestimated for those age groups born after the introduction of vaccination. Firstly, in the nonparticipation analysis restricted to those who filled out a questionnaire, individuals who considered none of the vaccinations necessary were less likely to participate. Secondly, for diphtheria, tetanus and poliomyelitis in the younger age groups, the estimated prevalence of antibodies exceeded the vaccination coverage of 97%. With respect to this last factor, a greater seroprevalence than vaccination coverage alone provides, could also be due to reaching detectable antibody levels after less than three vaccinations [note that vaccination

coverage is measured for 12-months-olds for at least three DT(P)-IPV] or by (additional) vaccinations given afterwards.
Although we cannot exclude the possibility that our population-based estimates were slightly overestimated as a result of overrepresentation of vaccinated individuals, the effect was probably very small and did not interfere with the interpretation of our results.

Vaccinated individuals were likely overrepresented among orthodox reformed individuals who participated in our study. Nonparticipants for whom questionnaire data were available reported less frequently that they had participated in the vaccination programme than participants. As no information on religion and vaccination status was available for nonparticipants who did not answer the questionnaire, the true nonparticipation selection is unknown. Even if the prevalence of diphtheria antitoxin antibodies, tetanus antitoxin antibodies and neutralising antibodies against poliovirus types 1, 2 and 3 estimated for these groups were overestimated, they were still were much smaller than those of the nationwide sample. They should probably be considered as upper limits.

Age-cohort effects

This type of fallacy quite often pops up in cross-sectional seroprevalence studies (1). The effect of age and cohort cannot be distinguished in such a study. The seroprevalence measured in a specific age group in a particular year does not imply that other individuals will have a similar prevalence of antibodies when they reach this age. For example, when the exposure to a pathogen decreases with time, younger birth cohorts will have a smaller prevalence of antibodies at the time they reach the same age group as the older birth cohorts in which the prevalence was measured. The prevalence estimates of hepatitis A antibodies in a cross-sectional study is affected by such a cohort effect for those born before World War II (much exposure) and those born after World War II (little exposure) as a result of changes in hygienic conditions.
For vaccine-preventable diseases, a cohort effect is observed in the antibody prevalence in individuals born before and after the introduction of vaccination. In our analysis of antibody levels of diphtheria, tetanus and poliomyelitis in 10 to 34-year-olds who were completely vaccinated according to the National Immunisation Programme standards, we interpreted the decrease in geometric mean titres longitudinally, that is, as a decrease with increasing time since the last vaccination. Tetanus antibodies are only induced after vaccination. It is very unlikely that individuals from the age groups concerned, those aged 10 to 34 years in the period 1995-1996, were exposed to poliovirus or *Corynebacterium diphtheriae*. Circulation of poliovirus during the outbreaks in 1978 and 1992-1993 seemed to be restricted to orthodox reformed groups refusing vaccination (Chapter 6) and only very few cases of diphtheria occurred after 1960 (Chapter 4).

For these reasons, we consider longitudinal interpretation of the decrease in geometric mean titres justified.

Validity of vaccination status

In studies evaluating the effects of national immunisation programmes, such as described in this thesis, vaccination status is a very relevant determinant. The validity of self-reported vaccination status was low in our pilot study (2). For example, many participants who reported to be completely vaccinated according to the National Immunisation Programme were not, or were incompletely vaccinated according to the vaccination certificate of this programme. For this reason we have collected data on vaccinations documented by the National Immunisation Programme, and/or military service and travel papers. Although such documentation is probably not completely infallible, it is highly unlikely that individuals with documented vaccinations did not receive them.
We restricted the analysis of the persistence of antibodies after vaccination to those individuals with complete documentation of vaccinations provided by the National Immunisation Programme. Individuals with documented revaccinations and those individuals without documentation who claimed to have been revaccinated were excluded from this analysis. It is possible that a few individuals in the subgroup might have forgotten that they had been revaccinated. If these revaccinations were not documented or if the individual did not show us a document of revaccination of some kind, this could have led to an underestimation of the decrease in geometric mean titres after complete vaccination.

Surveillance and seroepidemiology of pertussis

Validity of notification data

The most important problem of surveillance data concerns the validity. The number of patients reported will be influenced by many factors. If these factors remain constant, comparisons can be made to study changes in disease occurrence over time. However, if changes in these factors occur over time, the interpretation of surveillance data is hampered. We summarise the most important factors known to have influenced our surveillance data on pertussis.

Firstly, due to the increasing application of serology, the number of notifications increased in the 1980s. Immunoassays for measurement of IgA antibodies against *B. pertussis* and IgG antibodies against pertussis toxin became available in 1981 and 1984, respectively.
Secondly, changes in the interpretation of serology have influenced the number of pertussis notifications in various periods. Before 1988, the conclusion that serology was positive was

mostly based on the detectability of moderate or high titres in one serum sample. From 1988 onwards, according to notification criteria introduced in that year, serology was defined to be positive when an increase in titre in paired sera was detected. This is referred to as "positive two-point serology". In recent years in addition to positive two-point serology, the clinical pertussis diagnosis is confirmed by high titres in one serum sample. An increasing number of these patients with "positive one-point serology" was reported. The criteria for positive one-point serology are more strict than before 1988: higher titres have to be detected.
Another factor known to have influenced the number of reported pertussis cases is the notification rate. The notification rate has increased in recent years, apparently as a result of the pertussis outbreak in the period 1996-1997.

We could obtain insight into the influence of the factors just mentioned, since we were able to link the notification database with the serodiagnostic database of the National Institute of Public Health and the Environment. Therefore, we could rule out the possibility that the increase in incidence in 1996 was due to a change in serodiagnostic practice. Furthermore, we applied the present criteria for positive one-point serology retrospectively to serological test results in 1986 and 1987. We could see from the results of this that it was more likely that a true increase in pertussis incidence had occurred in 1986 and 1987.
As a result of a change in serodiagnostic practice and in the notification rate, comparison of the numbers of notifications at present with those before the pertussis outbreak in 1996 and 1997 is difficult.

The National Institute of Public Health and the Environment was the only laboratory that performed pertussis serology until 1998; since then other laboratories also do so. Furthermore, the law for notification has changed: now, some information necessary for linking the notification database to the serodiagnostic database is no longer available. Both factors make the surveillance and the interpretation of the data even more complicated.

Hospital admissions of pertussis patients are likely to be less sensitive to changes in the factors just described. Therefore, this source is very useful, not only for the interpretation of national surveillance data, but also for comparison between countries. Case definitions for notification, reporting and diagnostic practice differ greatly among the various countries. Differences in hospital admissions will probably vary less, particularly for young infants who suffer the disease most severely. However, since hospital admissions are restricted to these severe cases, we should not rely only upon this source.

Vaccine efficacy estimations

We used notification data to estimate vaccine efficacy with the screening method in Chapters 7 and 8 (3). Results obtained by the screening method may be biased towards either a higher or lower estimate of vaccine efficacy if the data source does not provide the true vaccination status of pertussis patients or pertussis vaccination coverage in the population under study (4).
Since the vaccination coverage is stable, according to the reliable registration system, we expect the estimate of the vaccine efficacy to be influenced only by reliability of the vaccination status (5). The reported cases are a selection of all pertussis cases. For example, pertussis vaccines appear to protect better against severe disease than against milder disease or asymptomatic infection with *B. pertussis* (6). Asymptomatic, subclinical and mild, unrecognized cases will not be reported. This may lead to underrepresentation of vaccinated patients. However, it is also possible that physicians in regions with low vaccination coverage, who often diagnose pertussis in unvaccinated children, have a lower notification rate.
Therefore, our objective was merely to study changes in vaccine efficacy. Similarly, as discussed for the validity of surveillance data, factors that influence reporting and are associated with vaccination status have to remain constant over time if we are to make comparisons over time and interpret changes in vaccine efficacy.
Our surveillance data show a decrease in vaccine efficacy over time. This decrease could have resulted from a change in the physician's perception and/or the method of diagnosis that could have caused selective reporting of vaccinated patients, a higher rate of misclassification of cases with respect to vaccination status than previously, and a change in the severity of the disease. However, the decrease could only be nullified assuming unlikely large changes in the selective reporting of vaccinated individuals or the rate of misclassification. Furthermore, the decrease could only partially be explained by a larger proportion of cases with positive one-point serology among the reported cases. These patients were more often vaccinated. Unfortunately, we could not verify what the effect of a change in the severity of disease would be. Ideally, a case control study to estimate the vaccine efficacy stratified by the severity of disease should have been performed. However, for practical reasons, this was not feasible. Instead, we collected data on symptoms and vaccination status of reported patients and hospitalized patients (7). These data show that typical pertussis also occurs among vaccinated individuals. It seems unlikely that cases that occurred before the epidemic were more severe than those reported in our study. Thus, it appears the decrease in vaccine efficacy at least partially reflects a true decrease.

Methodological issues in the study on high IgG-PT levels as a serodiagnostic tool for pertussis

IgG-PT distribution in the general population

The IgG-PT distribution measured in the nationwide population-based study seems to reflect the distribution in the population. The IgG-PT levels were not affected by differential response for those variables available for both nonparticipants and participants. Furthermore, even less effect on the IgG-PT distribution was expected from differential response by vaccination status than on the prevalence of antibodies for diphtheria, tetanus and poliomyelitis. At the ages of vaccination, only a very slight effect, or none at all, was observed on the IgG-PT distribution. Furthermore, since we show in our study that IgG-PT decreased after natural infection, it is likely that after vaccination IgG-PT levels will decrease.

IgG-PT in pertussis patients

We considered IgG-PT levels measured in second sera submitted to our serodiagnostic laboratory for those patients who showed at least a fourfold increase in IgG-PT, characteristic for the reconvalesence phase of those patients with a *B. pertussis* infection who showed an IgG-PT response. Ideally, we would have measured IgG-PT levels in sera collected from a random sample of patients starting at the moment of infection with *B. pertussis* to the highest IgG-PT level reached. However, such a study is not feasible. Our approach seems to be justified, but we cannot rule out the possibility that the selection of patients somehow differs in their IgG-PT response.

This issue also applies to the decrease in IgG-PT after infection, which we studied in follow-up sera of patients with clinical pertussis. Most of these patients were less than 10 years of age. Ideally, we would have collected sera from a random sample of patients rather than patients remaining under treatment of a physician, as was the case here. Furthermore, including patients of all ages would have made it possible to study more appropriately whether the decrease in IgG-PT depended on age.

Implications for public health and research

The major challenge in infectious disease control is eradication of the pathogen. Unless the pathogen is eradicated, continuous efforts are needed for disease control, even when the disease has been eliminated. The vaccine-preventable diseases described in this thesis seem to be in different phases of control in the Netherlands. Some are well on the way to eradication (poliomyelitis), or elimination (diphtheria and tetanus) and some, despite great progress, need more effective control (pertussis).

Here we discuss our findings with regard to possible implications for public health and research in making progress towards eradication, elimination or better control of poliomyelitis, diphtheria, tetanus and pertussis in the Netherlands and, if possible, worldwide.

Diphtheria

The implementation of immunisation has ensured that diphtheria has not been a public health problem for a generation in many industrialised countries. At the beginning of the 1990s, there was optimism that elimination of indigenous respiratory diphtheria could be achieved in the European Region by 1990 (8,9). The elimination of diphtheria is facilitated by the facts that humans are the only reservoir and that an effective, safe and inexpensive vaccine exists. Factors that might hinder the elimination of diphtheria are the presence of an asymptomatic carrier state and the need for three initial doses of vaccine and subsequent booster doses (9).

Our surveillance data show that diphtheria has virtually been eliminated in the Netherlands (10). However, diphtheria has re-emerged since 1990 on a massive scale in the New Independent States of the former Soviet Union (11,12). Among other contributing factors such as population movement, socioeconomic instability, and a deteriorating health infrastructure, a major reason for the epidemic is probably the presence of highly susceptible child and adult populations (12). Although a high rate of adult susceptibility might not be a sufficient precondition for the development of epidemic diphtheria in adults, it is considered a necessary condition (11). It is likely that the susceptibility of children is more important in the dissemination of diphtheria (11). The peak in measles following the school opening after vaccination exemplifies the role of contacts between children as a important determinant for the overall transmission potential (13).

In contrast to the situation in the former Soviet Union before the diphtheria outbreak, our population-based study shows that the diphtheria immunity level in the Dutch general population born after the introduction of vaccination is sufficient. This is the result of a successful vaccination programme with high vaccination coverage. However, gaps in immunity might exist among adults born before the introduction of vaccination. The prevalence of diphtheria antitoxin antibodies decreases sharply with increasing age. Similar findings are reported for other countries (14,15). This is evidence of the phenomenon of waning immunity after natural infection in the absence of boosting.

In specific orthodox reformed groups that refuse vaccination and that include many children and adults without detectable diphtheria antitoxin antibodies, the immunity level resembles the situation of great susceptibility in children and adults in the former Soviet Union.

The implications of these findings for public health and research depend on the risk of diphtheria spreading in the general population and among orthodox reformed individuals.
No link was established between two cases reported in the Netherlands since 1991 and Eastern Europe. Cases of diphtheria imported from the Newly Independent States have been reported in some other European countries (16). No secondary cases were reported following these imported cases (16). It was reported that the disease has become rare in the United States, and no outbreaks from secondary transmission were observed (17). Another favourable factor might be that the basic reproduction number of diphtheria (a measure of maximum transmissibility of the pathogen in a population) is smaller than that of other childhood diseases (18). This implies that the critical number of susceptible people required for spread of diphtheria is relatively large.
These observations are reassuring and suggest that the diphtheria immunity in the population of the Netherlands, as well as other countries, will suffice, despite the lack of antibodies in a large number of adults.

An outbreak of diphtheria in Mongola, affecting older children and young adults, is suspected to be a result of imported index cases (12,16). Two cases of diphtheria were diagnosed in one family in Denmark in 1998 (19). One of the patients was unvaccinated, while the other had likely received three vaccinations. Neither the patients, the family members, nor close contacts had a history of travel. It is suggested that one of the patients died of diphtheria, although it is not known how she became infected. Enhanced surveillance of patients with upper respiratory symptoms in a Northern Plains community in the United States strongly suggests long-term persistence of toxigenic *C. diphtheriae* in this region (20). These observations should alert us, although, or perhaps because, their meaning remains uncertain.

Such observations show the relevance of studying whether adults with low or undetectable antibody levels are sufficiently protected. Insight into the protection of these individuals could be obtained by offering diphtheria vaccination and by studying whether these individuals show an adequate (memory) response. Like others, our results indicate a good memory response after revaccination (21,22). However, the (memory) response to initial vaccination of adults whose naturally acquired antibodies have waned is unknown. If a large proportion of adults without detectable antibodies show a good memory response, it is likely that an adequate response will also occur after contact with *C. diphtheriae*. The National Health Council recommends studying the feasibility of vaccinating all adults with DT-IPV, but, at present, this has not been done (23).
Considering the great susceptibility in both children and adults among orthodox reformed groups and their sociogeographic clustering, there seems to be a real potential risk of spreading *C. diphtheriae* in these groups after the introduction of the bacterium (24). Lack of herd immunity resulted in a few outbreaks of poliomyelitis in these orthodox reformed groups (25). It is unclear why – fortunately – these groups have remained free of diphtheria.. Acceptance of vaccination in

these groups is not likely to improve considerably in near future. Although there are no signs of persistent circulation of toxigenic *C. diphtheriae* in our country, it would be worthwhile to assess this systematically for these unvaccinated orthodox reformed groups who are sociogeographically clustered. Specific media are needed for *C. diphtheriae* isolation; they are not routinely used unless the laboratory concerned is alerted that diphtheria is suspected (26).

Many factors involved in the occurrence of diphtheria remain obscure. Mathematical modelling may provide a little bit more insight into situations in which diphtheria cases can be expected. The role of susceptibility of children in addition to that of adults and the amount of import needed for spreading *C. diphtheriae* come to mind here. The standardised data on diphtheria antitoxin antibodies in various European countries in the European Sero-Epidemiology Network might be useful in this regard to compare countries with different immunity levels (27).

Tetanus

Tetanus is unique among the vaccine-preventable infectious diseases in that it is not communicable. It cannot be eradicated as a result of the ubiquity of spores in the environment. Furthermore, the resulting absence of herd immunity means that every unvaccinated individual is at risk of tetanus.

Our surveillance data show that about two or three cases of tetanus (0.16 per 1,000,000) have been reported each year in the Netherlands during the last two decades; an incidence similar to that in the United States (10,28). In the Netherlands almost all of these patients were born before 1945, which was before the introduction of vaccination. These surveillance data, together with the results of our study on tetanus antitoxin antibodies, show that the occurrence of tetanus is closely associated with the tetanus antitoxin antibody level in the population. Almost all the individuals without detectable antibodies were aged 50 years or more; in other words they were born before 1945.
The prevalence of tetanus antitoxin antibodies among individuals eligible for mass vaccination (those born after 1945) was very large. Antibody levels remain high for at least 20 years after complete vaccination in the National Immunisation Programme, but probably much longer. This implies that tetanus vaccine induces excellent protection.
These findings imply that in the case of an injury prescribing revaccination 5 years after previous vaccination in accordance with the guidelines of the World Health Organization instead of 1 year after previous vaccination - the current policy in the Netherlands - seems appropriate. Furthermore, offering a primary tetanus vaccination to unvaccinated individuals born before the introduction of vaccination is probably more effective in preventing tetanus than routine revaccination. Vaccination of these groups might be most efficient combined with other vaccines

(e.g. diphtheria). Incorporation in the regular influenza vaccination programme is a relevant option that could be studied.
To monitor the effect of such a scenario, information on the number of tetanus cases should be obtained. Reliable information on the number of tetanus cases has been obtained by the notification system until the present time. However, recently, notification of tetanus is no longer obligatory by law. Information on hospital admissions for tetanus has been shown to be invalid, probably due to misclassification of the diagnosis 'tetani' (10). As a result of lack of notification data on tetanus and since most cases of tetanus are diagnosed by the clinical symptoms and are not laboratory confirmed, an effort has to be made to make the surveillance of hospital admissions for tetanus more reliable. With a view to the small number of tetanus cases (but also of other vaccine-preventable diseases such as diphtheria) a strong appeal is made to physicians to recognize the clinical symptoms of a disease they have not seen before. Regular attention to the disease and the clinical symptoms in professional journals is helpful in this respect (26,29).

In developing countries, neonatal tetanus is still a serious problem. The World Health Organization estimates that the global number of neonatal tetanus deaths decreased from 408,000 in 1990 to 248,000 in 1997 (30). It is the second leading cause of death from vaccine-preventable diseases among children worldwide. The current goal is to eliminate neonatal tetanus (less than 1 case per 1000 live births) by the year 2000. Our study shows that tetanus vaccination gives very good protection, which indicates that the effectiveness of the vaccine is not the restrictive factor. However, since tetanus cannot be eradicated, continuous efforts have to be made to control this severe disease.

Poliomyelitis

In 1988, the goal of global poliomyelitis eradication by the year 2000 was adopted by the World Health Assembly (31). Eradication of poliomyelitis was considered feasible after the eradication of smallpox, one of the greatest public health achievements of all time (32). However, the differences between smallpox and poliomyelitis are great. Unlike smallpox, most infections with poliovirus are asymptomatic, and no sign is visible after infection or vaccination (33). Smallpox virus is less able to spread from person to person than poliovirus (34). However, the facts that humans are the only natural host of poliovirus, no long-term poliovirus carriers exist and the availability of effective vaccines (oral polio vaccine and inactivated polio vaccine) provide favourable conditions for eradication (33).
Great progress has been made towards eradication. Since the start of the initiative 10 years ago, the number of reported cases have fallen worldwide from more than 35,000 in 1989 to about 4000 in 1997. The Americas, are completely free of polio, and two other regions, the Western

Pacific and Europe, are close to being free (35-37). However, a recent outbreak of poliovirus type 3 in Angola clearly shows that the goal has not yet been achieved (38).

In the Netherlands, the last poliomyelitis cases occurred in an outbreak in the years 1992-1993 among orthodox reformed individuals, affecting 71 persons (10,25). No cases have been reported since then, and the surveillance of acute flaccid paralysis has brought no cases of poliomyelitis to light (10,39).

The results of our study on poliomyelitis immunity in the Netherlands show that all age groups are very well protected against poliomyelitis. Immunity seems to wane very little, after natural infection. This is more advantageous than diphtheria immunity; diphtheria antitoxin antibodies have decline in cohorts born before the introduction of vaccination.
Vaccination with inactivated polio vaccine seem to induce as many antibodies as natural infection. Again, this is more beneficial than the case for some other vaccine-preventable diseases. For example, vaccinated individuals were found to have fewer antibodies against measles and rubella than individuals with naturally acquired immunity. This may be one of the problems in measles elimination (40). For rubella, this may lead to an increased risk of congenital rubella syndrome when childbearing women must rely on vaccine-induced immunity obtained in childhood.

Unfortunately, the poliomyelitis immunity in sociogeographically clustered orthodox reformed individuals was much less favourable. Herd immunity in these groups might again turn out to be insufficient. Our results indicate that about half of the orthodox reformed individuals were infected during the outbreaks of 1978 and 1992-1993. This shows considerable spread of the virus among these groups, but since many individuals were not infected, hygiene measures might have had some effect in preventing the spread.
No poliomyelitis cases occurred outside the orthodox reformed groups during the outbreaks in the periods 1978 and 1992-1993. Although it had been shown that individuals vaccinated with inactivated polio vaccine could be re-infected with poliovirus, the herd immunity in the population was sufficient to prevent polio circulation outside the orthodox reformed groups (41). Lack of poliovirus circulation was indicated by the comparison of the seroprofiles for those born before and after the poliomyelitis outbreaks in the periods 1978 and 1992-1993.
Currently, the effect of challenge of oral polio vaccine (live attenuated virus) in elderly individuals is being studied. This will provide more evidence for the potential for poliovirus circulation in individuals vaccinated with inactivated polio vaccine (dead virus) and into the memory response of seronegative elderly persons. Information from this study can be used in mathematical modelling to determine how and when to stop polio vaccination in strategies for poliovirus eradication.

The implications of our results for the gaps in immunity of poliomyelitis, but also for diphtheria and tetanus, among orthodox reformed groups are limited. Mandatory vaccination is not considered an option, partly because this could result in a paradoxical effect of lower vaccination coverage in general. Moreover, changing the vaccine strategy from inactivated polio vaccine to oral polio vaccine is not recommended. Not only the risk of vaccine-associated paralytic poliomyelitis, but also the chance of infection of unvaccinated individuals is considered a disadvantage (42). A slight increase of vaccination coverage was observed for DT(P)-IPV in the province of Zeeland in cohorts born in the period 1991 to 1994, where many orthodox reformed individuals live (43). This increase was thought to be the result of greater acceptance of DT(P)-IPV of orthodox reformed individuals after the poliomyelitis outbreaks. Despite the secularisation in the Netherlands, no indications have been found that orthodox reformed individuals are less strict in refusing vaccination in general. Therefore, the effect is expected to be temporary. No further increase of the vaccination coverage was observed in cohorts born in 1995. Perhaps the initiative of offering vaccination to unvaccinated 16-year-olds, irrespectively of the reason for being unvaccinated, will have a beneficial effect on the vaccination coverage in this group (44). Furthermore, hygiene measures as recommended in the handbook "Care of poliomyelitis" and monitoring of poliovirus in sewage of schools with large numbers of orthodox reformed individuals might limit the spread of poliovirus when circulation occurs (42,45).

The findings of excellent protection after vaccination and the presence of herd immunity in the general population during outbreaks in orthodox reformed groups are important in view of the global initiative for polio eradication. The Netherlands is one of the few countries in which inactivated polio vaccine has been included in the vaccination programme from the beginning.
Most other countries use oral polio vaccine, and oral polio vaccine strains may continue to circulate. Before a region can be certified 'polio free', the World Health Organization demands the documented absence of wild type poliovirus in the population or its sewage. However, small amounts of wild type poliovirus are difficult to detect, particularly when circulation of oral polio vaccine strains occur (45).
Furthermore, vaccine-associated poliomyelitis cases will continue to occur. This could not only be disadvantageous for the patients involved, but also for the acceptance of vaccination in general. In the absence of circulation of wild poliovirus, such as in the United States, these are the only poliomyelitis cases occurring (46). Antivaccine media attention has grown, and vaccine-associated poliomyelitis has been a major issue (46). A decline in vaccination coverage is also very harmful for other vaccine-preventable diseases, such as is shown for pertussis and diphtheria (12,47).
The Dutch experience with long-term use of inactivated polio vaccine shows that the vaccine induces excellent protection. For all these reasons switching from oral polio vaccine to inactivated polio vaccine in the late stages of polio eradication might be advisable. It is now recommended that children receive IPV for the first two doses in the United States. Either IPV or OPV can be

administered for the third and fourth doses (48). An "IPV alone" policy will be implemented in 2000.

Pertussis

As for diphtheria, tetanus and poliomyelitis, more than 40 years of experience with pertussis vaccination exists. While the first three diseases have virtually been eliminated in the Netherlands, more effective control of pertussis is warranted. This is exemplified by the unexpected pertussis outbreak in the Netherlands in the period 1996-1997, which is reported in this thesis (Chapters 7 and 8). A resurgence of pertussis has also been reported in other countries such as the United States and Canada (49,50).

We have shown that changes in case definition and diagnostic practice may result in a greater number of reported pertussis cases. However, neither these changes, nor changes in vaccination coverage and vaccine quality, can explain the increased incidence of pertussis.
Two other factors, waning immunity and a change in *B. pertussis* strains, might play a role in the resurgence of pertussis. Following a discussion of their possible contributions some implications for vaccination strategy are discussed.

Data indicate that neither childhood immunisation nor infection provides long-term immunity against *B. pertussis*. Evidence is increasing that older children and adults are major reservoirs of infection due to waning immunity (51). It has been shown that these people play an important role in the transmission of infection to unvaccinated young infants, in which pertussis is most severe.
Pertussis in adults and older children was often overlooked, since the illness is often atypical and diagnosis is difficult. Diagnosis is initiated later in the disease, when cultures are unlikely to be positive (51). At this stage, serodiagnosis on a single serum sample facilitates the diagnosis of pertussis. We have found that high IgG-PT concentrations in a single serum sample are a specific and sensitive tool in pertussis diagnosis. Furthermore, our serological data show that detection of high IgG-PT levels in a single serum sample could confirm an increased number of pertussis cases, particularly for older age groups (Chapter 9). General practitioners and paediatricians should be on the alert, not only for the occurrence of pertussis in older (vaccinated) children and adults, but also for the possibility of confirming the diagnosis with one-point serology.

The decrease in vaccine efficacy observed in our surveillance data reflects at least a partly true decrease. We postulate that the increased incidence in the Netherlands might partially be caused by a mismatch between the vaccine strains and circulating strains of *B. pertussis*. The rather abrupt increase in the proportion of vaccinated patients of a wide age range.

A shift has occurred in the population structure of *B. pertussis*, and antigenic variants of pertactin and pertussis toxin of *B. pertussis* have emerged. They are distinct from the strains incorporated in the Dutch whole-cell vaccine (52-54). The analysis of strains from vaccinated and unvaccinated individuals indicated that the whole-cell vaccine protects better against strains with the vaccine type of pertactin than against strains with nonvaccine types (54). The role of pertactin in protection is indicated by a greater protection of a three-component acellular vaccine with pertactin (and PT and FHA) than of a two-component acellular vaccine (PT and FHA) in a Swedish trial (55). Furthermore, protection against clinical pertussis was found to be associated with pertactin antibodies (56,57).
The vulnerability of the population to antigenic changes in *B. pertussis* might be enhanced due to the small immunogenicity profile of the Dutch whole-cell vaccine (the vaccine induces low levels of IgG-PT). In response to the 1996-1997 outbreak, the amount of pertussis toxin in the vaccine was somewhat enhanced in November 1997.

Waning immunity indicates the need for booster vaccinations. Whole-cell vaccine was precluded from boosters because of the side effects. In response, modern acellular pertussis vaccines that are less reactogenic have been developed. The decisions on age at the time of boosting and on the type of vaccine (acellular or whole-cell) should be made carefully.
Our surveillance data show that cases from all ages were reported. The greatest incidence of pertussis, according to serological data and notification data, occurred at 4 years of age. Data on hospital admissions show a peak for infants less than 1 year old. Active surveillance of hospitalised children by paediatricians was started in 1997, and it has confirmed that pertussis is most severe among infants, who are too young to be vaccinated (less than 3 months of age) (7). The protection of these vulnerable infants has been the most important reason for pertussis vaccination. However, the paediatric surveillance and a study of the symptoms of reported cases shows that typical pertussis also occurs among vaccinated children, although very severe disease with complications is unlikely (7). Improving the protection of these children seems warranted.

On the short term, a booster in childhood is expected to prevent (vaccinated) children from becoming ill. It is also expected to prevent the disease among young infants by reducing the force of the infection. In response to the outbreak in the period 1996-1997 in the Netherlands, the National Health Council recommended a study of the effect of a booster vaccination at the age of 4 years (58). However, since the peak incidence occurred at the age of 4 years, a booster dose at an earlier age may be more effective.
The long-term epidemiological effect of a booster dose in childhood is unclear. If it postpones infection with *B. pertussis* to adult age, this might lead to a greater probability of transmission to young infants. Even with the advanced vaccination schedule that has been put into effect in 1999 (2, 3, 4 and 11 months instead of 3, 4, 5 and 11 months), the young infants can only be protected

by herd immunity. Maternal immunisation in pregnancy – if it were feasible – would probably be the most effective way of preventing pertussis among the youngest and most vulnerable infants (59). Similarly, maternal tetanus vaccination has been shown to be very effective in preventing neonatal tetanus.

The National Health Council recommends the use of an acellular vaccine for the booster dose (58). It is questionable whether the current acellular vaccines are the best choice for booster vaccination. Most of these acellular vaccines do not contain the antigenic variant of pertactin and pertussis toxin that dominates in the Netherlands. Regularly adapting the vaccines to circulating strains, as is done each year for influenza, might solve such a problem.
However, acellular vaccine contains a limited number of components. When the change in a component is only a marker for the true immunological change elsewhere in the bacterium, adaptation of the whole-cell vaccine that contains whole dead bacteria might be more effective. However, to maintain the wide acceptance of vaccination by the population, the adverse events that occur when a booster with whole-cell vaccine is given, should be reduced.

It is likely that a decision will soon be made on the booster dose in childhood. To study the short and long-term epidemiological effects of a such booster dose, routine surveillance should be continued. As described previously, this will be more complicated because of the decentralization of serology and the change in the law regarding notification. More insight into the major reservoirs of *B. pertussis* infection, particularly for young infants must be obtained. These reservoirs may change after the introduction of booster vaccination. The IgG-PT distribution in the general population (Chapter 9) in relation to the decline in IgG-PT antibody levels after infection makes it possible to study the frequency of *B. pertussis* infections in various age groups. The IgG-PT distribution suggests that *B. pertussis* infections occur frequently. This is exemplified by the increase in IgG-PT from 4 years of age onwards. The constant IgG-PT level for individuals 15 years old and older suggests that the increases in IgG-PT in infected individuals is in balance with the following decline in IgG-PT in other individuals. Differences in IgG-PT distributions in various European countries as collected in the European Sero-Epidemiology Network might be helpful in understanding the epidemiology of pertussis (27).
Furthermore, transmission studies should be directed to potential sources of infection for young infants. In this way, information can be obtained for more effective control of pertussis.

General conclusions

The studies in this thesis confirm the great success of the vaccination programme in the Netherlands. The challenge is, first of all, to preserve and enhance this progress by sustaining high acceptance of vaccination in childhood. As a result of the enormously decreased incidence of

vaccine-preventable diseases, the benefits of immunisation might gain less attention, and the focus might turn to real and perceived risks of vaccination. Therefore, it is extremely important to maintain the trust in vaccinations. For vaccines that give suboptimal, but still very good, protection, such as that for pertussis, this might be even more difficult. Physicians are inexperienced with the diseases as a result of low incidence and have to rely on education alone. They should be aware of the continuous need for vaccination and pay attention to giving good information to parents.

Furthermore, our studies show that we have to anticipate some long-term effects of mass vaccination. As a result of decreased circulation of the pathogens, gaps in immunity to diphtheria and poliomyelitis among orthodox reformed individuals were observed. A waning of natural-acquired antitoxin antibodies against diphtheria as a result of lack of boosting is shown. Inadequate levels of tetanus vaccination resulted in susceptibility of adults born after the introduction of vaccination and explain why most tetanus cases occur in these unvaccinated cohorts. In contrast to diphtheria antibodies, poliovirus antibodies did not wane or wane very little, after vaccination and natural infection. Waning pertussis immunity after infection and vaccination was reflected in the data on surveillance and seroepidemiology of pertussis. The pertussis data support the hypothesis of a mismatch of vaccine strains with the circulating strains, which might have occurred as a result of long-term pertussis vaccination.

These findings imply a need to adapt future vaccination strategies. They could prove relevant for countries and vaccine-preventable diseases outside the scope of this thesis.
We conclude that vaccination for diphtheria, tetanus and pertussis must no longer be directed only towards children, but also towards adults. The increased number of elderly people in general stresses the need for a more common practice of adult vaccination. The feasibility and positive effects are exemplified by the influenza programme directed towards individuals aged 65 years and older, which is carried out by general practitioners.
The Dutch experience of excellent immunity after vaccination with inactivated polio vaccine is an example where other countries can partly draw on to decide on change in vaccination strategy. Particularly when randomised controlled trials are not feasible, for example due to very great vaccination coverage, this may prove efficient.
Adaptation of vaccines to circulating strains that might be useful for pertussis immunisation, has already been proven effective for influenza.

The results of the studies in this thesis clearly show the role of seroepidemiology and surveillance in the evaluation and success of the National Immunisation Programme. Epidemiological studies to assess disease incidence and the immunity of the population will have to continue to evaluate the (long-term) epidemiological effects of the existing vaccines and new vaccines.

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Summary

Vaccination has been one of the most effective interventions in public health. However, despite great successes, even in a country with high vaccination coverage like the Netherlands, epidemics of some vaccine-preventable diseases still occur, and re-emergence of others is possible. The success of a vaccination programme can be improved by continuous evaluation after the licensing of a vaccine. Epidemiological methods play an important role in this evaluation to assess, the disease incidence and the immunity of the population, in addition to the safety of the vaccine and vaccine coverage.

Reduction in disease incidence achieved after a vaccine has been introduced is the best measure of the success of a programme. With fewer cases as a result of an effective programme, the role of serological surveillance becomes more evident. Assessment of specific antibodies offers an opportunity to identify groups with little immunity that might require changes in vaccination strategy to prevent (re)emergence of disease.

In this thesis, several aspects of surveillance as part of the evaluation of the Netherlands immunisation programme were described. These aspects include both disease surveillance (of pertussis) and immunosurveillance (of diphtheria, tetanus and poliomyelitis). Furthermore, the possibilities of improving pertussis serodiagnosis were studied. The aim was to find a more sensitive diagnostic tool for patient and laboratory surveillance.

The results are mainly based on two studies. In the first study, a population-based serum bank was established by collecting sera from the general population and from orthodox reformed groups refusing vaccination. In the second study, data on the number of pertussis patients from various surveillance sources were analyzed.

Immunosurveillance is one of the tools for studying the change in epidemiologic dynamics of infectious diseases as a result of mass vaccination (**Chapter 2**). The weaker force of infection can result in an increase of the mean age at the time of infection and a lack of boosting opportunities of both natural and vaccine-induced immunity. Clustering of unvaccinated individuals might lead to gaps in immunity in these groups. Herd immunity might break down, and epidemics can occur as a result. In the Netherlands, this could happen to orthodox reformed groups who reject vaccination on religious grounds and are sociogeographically clustered.

In order to estimate the immunity of the Dutch population, a population-based serum bank was established in the period 1995-1996. In a nationwide sampling, 8359 sera were collected (response rate 55%), and in a sample from municipalities with low vaccine coverage 1589 sera (response rate 52.5%) were collected. The sera from the latter sample were collected to gain access to orthodox reformed individuals refusing vaccination. In contrast to collecting residual sera from laboratories, this approach provided an opportunity for collecting extensive information on determinants of the immune status by means of a questionnaire.

Information from both participants and nonparticipants was used to identify characteristics of individuals associated with (non)participation in our population-based serum collection (**Chapter 3**). Five groups were distinguished: 7904 initial participants who gave blood and completed the questionnaire, 455 additional participants who gave blood during extra opening hours and completed the questionnaire, 1618 nonparticipants who completed the questionnaire, 1053 nonparticipants who completed a nonresponse questionnaire, and 4159 absolute nonparticipants for whom only data from the municipal registry was available. In dichotomous and polytomous logistic regression, the following variables were associated with (non)participation: age group, gender, marital status, nationality, kind of reminder, and degree of urbanisation. Some variables affected each form of (non)participation differently. E.g., comparing nonparticipants who completed the questionnaire with initial participants yielded an odds ratio of 3.5 for boys 0-4 year olds compared to men 30-64 years old, while the odds ratio was 0.5 comparing absolute nonparticipants with initial participants. Other variables affected each form similarly. E.g., comparing additional participants with initial participants, or each of the different forms of nonparticipation groups with initial participants yielded odds ratios for unmarried individuals compared to married individuals of about 1.5.
We concluded that investigators have to be aware that including additional participants might not always reduce nonparticipation selection and that studying only a subgroup of nonparticipants might lead to biased insight into nonparticipation selection.

To identify groups with little immunity, specific antibodies to diphtheria, tetanus and poliomyelitis that correlate with protection were assessed for individuals from the nationwide sample and from the low vaccine coverage sample that included orthodox reformed individuals.
Diphtheria antitoxin and tetanus antitoxin antibodies were measured in a toxin inhibition assay (**Chapters 4 and 5**). Neutralising antibodies against poliovirus types 1, 2 and 3 were measured in the neutralisation assay (**Chapter 6**). The effects of differential response of variables available for both participants and nonparticipants (**Chapter 3**) on population estimates for both the nationwide sample and the low vaccine coverage sample amounted to less than one standard error. They were therefore ignored.
In the nationwide sample, 58% had diphtheria antitoxin levels of at least 0.1 IU/ml; 30%, between 0.01 and 0.1 IU/ml; and 12%, less than 0.01 IU/ml. In this sample, 72.5% had tetanus antitoxin antibodies of at least 0.01 IU/ml, 14% between 0.01 and 0.1 IU/ml and 14% of less than 0.01 IU/ml. Neutralising antibodies (titre at least 1:8) against poliovirus types 1, 2, and 3 were measured in 97%, 93% and 90% of all individuals, respectively.
The age-specific prevalence of diphtheria antitoxin antibodies (≥ 0.01 IU/ml), of tetanus antitoxin antibodies (≥ 0.01 IU/ml) and of neutralising antibodies against poliovirus types 1 and 2 (≥ 1:8) in our nationwide sample was greater than 92.5% for individuals younger than 45 years. These individuals were born after the introduction of mass vaccination. For poliovirus type 3, the

prevalence of neutralising antibodies was greater than 90% for younger than 30 years and greater than 80% for 30 to 44-year-olds.
The antitoxin antibody levels for diphtheria and tetanus decreased sharply after 44 years of age, i.e. for those born before the introduction of vaccination. More than 50% of the 70 to 79-year-olds had diphtheria and/or tetanus antitoxin antibody levels below 0.01 IU/ml. For poliomyelitis, the prevalence of neutralising antibodies for all three types remained at 80% or more in the older age groups.
For individuals aged 10 to 34 years who were completely vaccinated according to the National Immunisation Programme standards (i.e. at the ages of 3, 4, 5, and 11 months, 4 and 9 years), the geometric mean titres decreased with increasing age. However, the prevalence of antibodies in this subgroup was still more than 98% for diphtheria antitoxin levels, for tetanus antitoxin levels of at least 0.01 IU/ml and for neutralising antibodies of poliovirus types 1 and 2. It was at least 94% for poliovirus type 3. The decline in geometric mean titres with age was similar for diphtheria antitoxin antibodies and tetanus antitoxin antibodies. Since the geometric mean titres were higher for tetanus than for diphtheria for recently completely vaccinated 10 to 14-year-olds, the tetanus antitoxin antibody levels were higher for 30 to 34-year-olds than the levels for diphtheria. The geometric mean titre for poliomyelitis decreased only slightly with increasing age. For all three diseases, the geometric mean titres for those individuals aged 20 to 34 years with evidence of revaccination were comparable to the titres of 10 to 14-year-olds who were completely vaccinated, but without evidence of revaccination.
Only 40% of the orthodox reformed persons had diphtheria and/or tetanus antitoxin antibodies of at least 0.01 IU/ml, while 65%, 59% and 69% had neutralising antibodies against poliovirus type 1, 2 or 3, respectively. The seroprofiles of orthodox reformed participants, but not of other participants, showed an effect of recent poliomyelitis outbreaks.
We concluded that the Dutch immunisation programme induced long-term diphtheria, tetanus and poliomyelitis immunity. While adults are very well protected against poliomyelitis, a great number of adults lack diphtheria or tetanus antitoxin antibodies. These adults might benefit from diphtheria (re)vaccination; however, with the prevention of tetanus in mind, offering a primary tetanus vaccination to cohorts born before the introduction of vaccination would probably be more effective than routine revaccination. Introduction of *Corynebacterium diphtheriae* or poliovirus in sociogeographically clustered orthodox reformed groups might constitute a substantial danger of spreading of these pathogens. Since the Netherlands pursues non-mandatory vaccination, global eradication such as envisioned for poliovirus, is the only way to protect these orthodox reformed groups.

While diphtheria, tetanus and poliomyelitis have virtually been eliminated from the Netherlands, better control for pertussis is warranted. Pertussis remains endemic, and epidemic peaks occur. Surveillance data of pertussis were analysed to obtain insight into the occurrence of pertussis and

possible factors involved in its re-emergence (**Chapters 7 and 8**). Besides notification data from 1989 to 1996 (**Chapter 7**), data from positive serodiagnosis, hospital admissions and deaths were collected for 1997 and earlier years (**Chapter 8**). In the period 1996-1997, a sudden increase in the number of pertussis cases was observed from notifications, positive two-point serology, positive one-point serology and hospital admissions. Comparing the average annual incidence in the period 1989 to 1995, the incidence in 1996 increased 12-fold according to notifications (2.3 versus 27.3 per 100,000), 6-fold according to cases with positive two-point serology (2.1 versus 12.2), 6-fold according to positive one-point serology (8.2 versus 50.7) and 3-fold according to hospital admissions (1.2 versus 3.3). Both in the period 1989 to 1995 and in 1996, two deaths were reported.

In 1996, according to notifications and serology data, the increase in pertussis incidence among (mostly unvaccinated) children less than 1 year of age was similar to the increase in hospital admissions. In older, mostly vaccinated, individuals, the increase of hospital admissions was relatively small. The increase of reported cases of vaccinated individuals of all ages was larger than that among unvaccinated individuals. The relatively greater increase in reported cases for vaccinated patients than for unvaccinated patients has already begun in 1994 and 1995, that is, before the sharp increase in the overall number of cases. The proportion of reported cases with positive two-point serology increased from 24% in 1993 to 42-43% in 1996 and 1997. For positive one-point serology, a sharp increase occurred in the proportion that was reported; from 6% in 1993 to 29% in 1997. This increase in the notification rate and the greater number of cases reported with positive one-point serology could not explain the sudden rise in pertussis cases. We conclude that the increased incidence, as well as the decrease in vaccine efficacy, reflected a partly real change. Furthermore, due to a mismatch between the vaccine strain and circulating *Bordetella pertussis* strains, we postulate that the proportion of pertussis infections resulting in recognizable symptoms has increased among vaccinated individuals. Perhaps the small immunogenicity profile of the Dutch vaccine has resulted in greater vulnerability of the population to antigenic *B. pertussis* changes.

Laboratory confirmation of pertussis by culture, polymerase chain reaction (PCR), or detection of antibody increase in paired sera is hampered by low sensitivity. Therefore, we investigated whether, and at which level, concentrations of IgG antibodies against pertussis toxin (IgG-PT) in a single serum sample are indicative of actual or recent pertussis (**Chapter 9**). IgG-PT, measured by ELISA in U per millilitre (ml), was analysed in 7756 population-based sera, in sera of 3491 patients with at least a fourfold increase of IgG-PT, in paired sera of 89 patients with positive cultures and/or PCRs, and in sera of 57 patients with clinically documented pertussis with a median follow-up of 1.4 years. Levels of IgG-PT of at least 100 U/ml were present in less than 1% of the participants in the population-based study. In other words, this gives a specificity of 99% for positive one-point serology based on this cut-off level. Such levels were present in more

than 80% of the sera of patients with clinical pertussis symptoms with at least a fourfold increase in IgG-PT in paired sera. The follow-up sera of patients with clinically documented pertussis showed that the IgG-PT level had decreased below 100 U/ml in more than 80% of the patients within 1 year after the onset of symptoms. The sensitivity of an IgG-PT level of at least 100 U/ml amounted to 76% in patients with positive pertussis PCR or culture. The number of patients with IgG-PT levels of at least 100 U/ml in a first serum sample in the serodiagnostic database was more than four times greater than the number of patients with positive two-point serology. This varied from onefold for 0 to 5-month-olds to 14-fold for individuals aged 15 years or more.
We conclude that, IgG-PT levels of at least 100 U/ml are a specific and sensitive tool for diagnosing a recent or actual *B. pertussis* infection. Diagnosis on the basis of a single serum sample could particularly facilitate the pertussis diagnosis in older children and adults because laboratory testing for these children and adults is initiated at a later stage of the disease. Therefore, this tool is helpful, not only for the individual patient, but also for epidemiological studies.

In the general discussion (**Chapter 10**), the methodological issues of selective participation, the age-versus-cohort effect and the validity of vaccination status for interpreting the results of the population-based studies, are discussed. Regarding the interpretation of pertussis surveillance data, validity of notification data and vaccine efficacy with the screening method were described. For the study on IgG-PT levels in single serum samples as a diagnostic tool in pertussis, methodological issues such as the validity of using IgG-PT levels of patients with at least a fourfold increase in titre as characteristic for reconvalenscence sera were discussed.

Implications for public health and research of the study findings were discussed with regard to potential progress in (maintaining) control of the diseases. The diphtheria epidemic in the former Soviet Union has shown that this disease can make a comeback in susceptible populations. The lack of diphtheria antitoxin antibodies in many adults as a result of waning immunity implies the need to study the (memory) response of adults who have low or undetectable antibody levels. Such a study would resolve the question whether people have sufficient immunity after exposure to *C. diphtheriae*. Tetanus vaccination induces excellent immunity, showing that prevention of tetanus could be made possible by directing vaccination to unvaccinated individuals. Primary vaccination of unvaccinated adults born before the introduction of mass vaccination seems to be the most effective method.
The Dutch experience with long-term use of inactivated polio vaccine shows that the vaccine induces excellent protection, as well as the ability to maintain herd immunity. Therefore, to prevent vaccine-associated poliomyelitis, changing from oral polio vaccine to inactivated polio vaccine might be advisable in the late stages of global poliovirus eradication.

Potential interventions with respect to gaps in diphtheria, poliomyelitis and tetanus immunity among orthodox reformed groups are limited. Monitoring the occurrence of the circulation of the pathogens, would detect (re)emergence of the diseases as early as possible.
Progress towards better control of pertussis could be made by implementing booster vaccination after infant vaccination. Adaptation of such a vaccine to circulating strains might also be effective. Both the choice of the most appropriate vaccine and the age of boosting are complicated as a result of lack of insight into long-term effects on the pertussis epidemiology. Not only surveillance data, but also data on the major reservoirs for infection, particularly for unvaccinated young infants, are needed to monitor the effect on pertussis epidemiology.

In general, our results show that the high degree of acceptance of vaccination for children should be sustained, and that vaccination should also be directed towards adults. Adaptation of vaccines to circulating strains might be useful for pertussis, as has already been proven effective for influenza.
In order to monitor changes in epidemiology of vaccine-preventable diseases after vaccination, epidemiological studies such as the ones described in this thesis will have to be repeated periodically in order to improve the success of (new) vaccination programmes.

Samenvatting

Vaccinatie is een van de meest effectieve interventies bij het bevorderen van de volksgezondheid. Toch komen nog steeds epidemieën voor van ziekten waartegen gevaccineerd wordt, zelfs in een land met een hoge vaccinatiegraad zoals Nederland. Ook is het niet uitgesloten dat andere ziekten waartegen gevaccineerd wordt weer zullen gaan optreden. Het succes van een vaccinatie programma kan worden verbeterd door continue evaluatie na introductie van een vaccin. Epidemiologische methoden spelen hierbij een belangrijke rol; niet alleen om de veiligheid van een vaccin en de vaccinatiegraad vast te stellen, maar ook om de incidentie van de ziekte en immuniteit in de bevolking te bepalen.
Het succes van een programma na introductie van een vaccin kan goed worden aangetoond door het vaststellen van een daling van de ziekte waartegen wordt gevaccineerd. Wanneer door een effectief programma het aantal ziektegevallen is verminderd, wordt de rol van serologische surveillance duidelijker. Door het bepalen van specifieke antistoffen kunnen groepen met lagere immuniteit worden opgespoord. Op grond hiervan kunnen veranderingen in vaccinatiestrategie noodzakelijk blijken om het (weer) optreden van de ziekte te voorkomen.
In dit proefschrift zijn diverse aspecten van surveillance als onderdeel van de evaluatie van het Nederlandse vaccinatieprogramma beschreven. Deze betreffen zowel surveillance van ziekte (ten gevolge van kinkhoest) als immunosurveillance (van difterie, tetanus en poliomyelitis). Ook werd onderzocht of serodiagnostiek van kinkhoest kon worden verbeterd. Een sensitievere diagnostiek methode voor kinkhoest is zowel voor de individuele patiënt als voor laboratorium surveillance waardevol.
De resultaten zijn hoofdzakelijk gebaseerd op twee onderzoeken. In de eerste studie is een serumbank opgezet van de Nederlandse bevolking waarbij sera zijn verzameld van de algemene bevolking en van orthodox gereformeerde personen die vaccinatie afwijzen. In het tweede onderzoek werden gegevens van kinkhoest patiënten uit diverse surveillance bronnen geanalyseerd.

Immunosurveillance is een van de methoden waarmee veranderingen in epidemiologische dynamiek van infectieziekten na routine vaccinatie bestudeerd kan worden (**Hoofdstuk 2**). Door de lagere infectiedruk kan de gemiddelde leeftijd waarop infectie optreedt, toenemen. Natuurlijke en vaccin- geïnduceerde immuniteit kunnen minder goed worden onderhouden. Clustering van ongevaccineerden kan leiden tot een groot aantal vatbare individuen binnen zulke ongevaccineerde groepen. Als hierdoor de groepsimmuniteit wordt doorbroken kunnen epidemieën optreden. In Nederland kan dit voorkomen bij orthodox gereformeerde groepen die vaccinatie afwijzen op grond van hun religie omdat zij in sociaal en geografisch opzicht zijn geclusterd.

Om inzicht te krijgen in de immuniteit van de Nederlandse bevolking werd in 1995-1996 een serumbank van de algemene bevolking opgezet. In een nationale steekproef zijn 8359 sera verzameld (respons 55%); in een steekproef van gemeenten met een lage vaccinatie graad 1589 sera (respons 52.5%). De sera uit de laatste steekproef zijn verzameld om toegang te hebben tot orthodox gereformeerden die vaccinatie afwijzen. In tegenstelling tot residuele sera van laboratoria, kon met deze benadering uitgebreide informatie over determinanten van immuunstatus worden verzameld met behulp van een vragenlijst.

Informatie over deelnemers en niet-deelnemers is gebruikt om karakteristieken te identificeren, die geassocieerd waren met (niet)deelname aan onze serum verzameling van de bevolking (**Hoofdstuk 3**). Vijf groepen zijn onderscheiden: 7.094 initiële respondenten gaven bloed en vulden de vragenlijst in; 455 additionele respondenten gaven bloed tijdens een extra spreekuur en vulden de vragenlijst in; 1.618 nonrespondenten vulden alleen de vragenlijst in; 1.053 nonrespondenten vulden een nonrespons vragenlijst in, en van 4.159 absolute nonrespondenten was alleen informatie uit het bevolkingsregister beschikbaar. In dichotome en polychotome logistische regressie waren de volgende variabelen geassocieerd met (non)participatie: leeftijd, geslacht, burgerlijke staat, nationaliteit, soort van herinnering en mate van urbanisatiegraad van de gemeente. Sommige variabelen hadden een verschillende invloed op de diverse vormen van (non)participatie. Bijvoorbeeld, vergelijking van nonrespondenten die alleen de vragenlijst invulden met initiële respondenten leverde een odds ratio van 3,5 voor mannen van 0-4 jaar versus mannen van 30-64 jaar, terwijl voor de vergelijking van absolute nonrespondenten met initiële respondenten deze odds ratio 0,5 bedroeg. Andere variabelen beïnvloedden elke vorm van (non)participatie overeenkomstig, Zo bedroegen de odds ratios voor ongehuwden versus gehuwden ongeveer 1,5 zowel voor de vergelijking van additionele respondenten met initiële respondenten als voor de vergelijkingen van elk van de drie groepen van nonrespondenten met initiële respondenten.
We concludeerden dat onderzoekers zich er van bewust moeten zijn dat het opnemen van additionele participanten niet altijd leidt tot een vermindering van nonparticipatie selectie. Ook het bestuderen van een subgroep van nonparticipanten kan een verkeerd beeld geven van deze selectie.

Specifieke antistoffen die correleren met bescherming tegen difterie, tetanus en poliomyelitis werden bepaald voor deelnemers uit de nationale steekproef en voor deelnemers uit de steekproef van gemeenten met een lage vaccinatiegraad. In de laatste steekproef waren orthodox gereformeerde personen opgenomen.
Difterie antitoxine en tetanus antitoxine antistoffen zijn gemeten met een toxine inhibitie test (**Hoofdstuk 4 en 5**). Neutraliserende antistoffen tegen poliovirus type 1, 2 en 3 zijn bepaald in de neutralisatie test (**Hoofdstuk 6**). Het effect van verschillende kansen op deelname voor variabelen

die voor participanten en nonparticipanten beschikbaar waren, bedroeg minder dan een standaard fout voor de populatie schattingen van beide steekproeven. Dit effect werd daarom genegeerd.
In de nationale steekproef had 58% van de deelnemers difterie antitoxine antistoffen van tenminste 0.1 IU per ml, 30% van 0.01 tot 0.1 IU per ml en 12% van minder dan 0.01 IU per ml. In deze steekproef had 72.5% van de deelnemers tetanus antitoxine antistoffen van tenminste 0.01 IU per ml, 14% van 0.01 tot 0.1 IU per ml en 14% van minder dan 0.01 IU per ml. In 96%, 93% en 90% werden neutraliserende antistoffen (titer ≥ 1:8) tegen respectievelijk poliovirus type 1, 2 en 3 gemeten.
De leeftijdsspecifieke prevalentie voor personen van jonger dan 45 jaar bedroeg voor difterie antitoxine antistoffen (≥0.01 IU per ml), voor tetanus antitoxine antistoffen (≥0.01 IU per ml) en voor neutraliserende antistoffen tegen poliovirus type 1 en 2 tenminste 92.5%. Deze personen zijn geboren na de introductie van routine vaccinatie. De prevalentie van neutraliserende antistoffen voor poliovirus type 3 was hoger dan 90% voor degenen jonger dan 30 jaar en hoger dan 80% voor personen in de leeftijd van 30 tot 44 jaar.
De difterie en tetanus antitoxine antistoffen namen sterk af na de leeftijd van 44 jaar; dat wil zeggen voor degenen die geboren waren voor de introductie van routine vaccinatie. Meer dan vijftig procent van 70 tot 79 jarigen had difterie en / of tetanus antitoxine antistoffen van minder dan 0.01 IU per ml. Voor poliomyelitis bleef de prevalentie van neutraliserende antistoffen in oudere leeftijdsgroepen voor de drie typen 80% of hoger.
De geometrisch gemiddelde titers namen af met de leeftijd voor personen van 10 tot 34 jaar die compleet gevaccineerd waren volgens het Rijksvaccinatieprogramma (dat wil zeggen op de leeftijden van 3, 4, 5 en 11 maanden, 4 en 9 jaar). De prevalentie van antistoffen in deze subgroep was tenminste 98% voor difterie antitoxine antistoffen, tetanus antitoxine antistoffen en neutraliserende antistoffen tegen poliovirus type 1 en 2. Voor poliovirus type 3 bedroeg deze 94%. De afname in geometrisch gemiddelde titer was vergelijkbaar voor difterie en tetanus. Doordat de geometrisch gemiddelde titers voor recent gevaccineerde 10 tot 14 jarigen voor tetanus hoger waren dan voor difterie, lagen deze niveau's voor tetanus voor 30 tot 34-jarigen boven die voor difterie. Voor poliomyelitis was de afname in geometrisch gemiddelde titer gering. De geometrisch gemiddelde titers voor die deelnemers van 20 to 34 jaar met bewijs van revaccinatie, was voor de drie ziekten vergelijkbaar met de geometrisch gemiddelde titers van recent gevaccineerde 10 tot 14 jarigen zonder enig bewijs van revaccinatie.
Slechts 40% van orthodox gereformeerde personen had difterie en/of tetanus antitoxine antistoffen van tenminste 0.01 IU per ml, terwijl respectievelijk 65%, 59% en 69% neutraliserende antistoffen had tegen poliovirus type 1, 2 of 3. Voor orthodox gereformeerden is, in tegenstelling tot de overige deelnemers, in de seroprevalentie curven een effect te zien van de recente poliomyelitis epidemieën.
We concludeerden dat het Rijksvaccinatieprogramma langdurige bescherming induceert tegen difterie, tetanus en poliomyelitis. Volwassenen zijn goed beschermd tegen poliomyelitis, maar een

aanzienlijk deel heeft geen antistoffen tegen difterie of tetanus toxine. Deze volwassenen zouden kunnen profiteren van (re)vaccinatie tegen difterie. Om ziektegevallen ten gevolge van tetanus te voorkomen, lijkt het effectiever om een primaire tetanus vaccinatie aan te bieden aan cohorten geboren voor de introductie van vaccinatie dan om routine revaccinatie in te voeren. Introductie van *Corynebacterium diphtheriae* of van poliovirus in orthodox gereformeerde groeperingen die in sociaal en geografisch opzicht zijn geclusterd, zou mogelijk tot verspreiding van deze pathogenen kunnen leiden. Aangezien Nederland vrijwillige vaccinatie nastreeft, zou wereldwijde eradicatie zoals wordt voorzien voor poliovirus, de enige manier zijn om deze orthodox gereformeerde groepen te beschermen.

Terwijl difterie, tetanus en poliomyelitis vrijwel uit Nederland zijn geëlimineerd, is betere beheersing van kinkhoest nodig. Kinkhoest blijft endemisch met epidemische pieken. Om inzicht te krijgen in het voorkomen van kinkhoest en in factoren die betrokken zouden kunnen zijn bij het weer opkomen van kinkhoest, zijn surveillance gegevens geanalyseerd (**Hoofdstuk 7 en 8**). Behalve gegevens uit aangiften wegens kinkhoest van 1989 tot 1996 (**Hoofdstuk 7**), zijn gegevens van positieve serodiagnostiek, ziekenhuisopnamen en overledenen verzameld van 1997 en eerdere jaren (**Hoofdstuk 8**). In 1996-1997 nam het aantal kinkhoest patiënten op basis van aangiften, bevindingen van positieve tweepuntsserologie, bevindingen van positieve eenpuntsserologie en ziekenhuisopnamen plotseling toe. In vergelijking tot de gemiddelde jaarlijkse incidentie in de periode 1989 tot 1995, nam de incidentie in 1996 volgens aangiften twaalfvoudig toe (2,3 versus 27,3 per 100.000), zesvoudig volgens patiënten met positieve tweepuntsserologie (2,1 versus 12,2), zesvoudig volgens patiënten met positieve eenpuntsserologie (8,2 versus 50,7) en drievoudig volgens ziekenhuisopnamen (1,2 versus 3,3). Zowel van 1989 tot en met 1995 als in 1996 werden twee overledenen ten gevolge van kinkhoest gerapporteerd.
De toename in de kinkhoest incidentie in 1996 op grond van aangiften en serologische gegevens was voor (grotendeels ongevaccineerde) kinderen jonger dan een jaar vergelijkbaar met de toename in ziekenhuisopnamen. Voor oudere, grotendeels gevaccineerde personen, was de toename in ziekenhuisopnamen relatief klein. De verhoging van het aantal aangegeven patiënten was groter voor gevaccineerden dan voor ongevaccineerden. De grotere toename in gevaccineerde aangegeven patiënten ten opzichte van ongevaccineerde aangegeven patiënten begon reeds in 1994-1995, dat wil zeggen voordat de verheffing in het totaal aantal patiënten werd waargenomen. Het aandeel patiënten met positieve tweepuntsserologie dat werd aangegeven nam toe van 24% in 1993 tot 42-43% in 1996 en 1997. Voor positieve eenpuntsserologie nam het aandeel dat werd aangegeven sterk toe; namelijk van 6% in 1993 tot 29% in 1997. Deze verhoging in aangifte discipline en het groter aantal patiënten dat op grond van eenpuntsserologie werd aangegeven, kon de plotselinge toename in het aantal kinkhoest patiënten niet verklaren. We concludeerden dat de toegenomen incidentie en de verlaagde vaccin-

effectiviteit voor een deel werkelijke veranderingen reflecteerden. Ook veronderstelden we dat ten gevolge van een mismatch tussen vaccin stammen en circulerende *B. pertussis* stammen, het aandeel van de kinkhoest infecties onder gevaccineerden met herkenbare symptomen, groter is geworden. Misschien dat het smalle immunogeniciteitsprofiel van het Nederlandse vaccin een grotere gevoeligheid van de bevolking te weeg heeft gebracht voor antigene veranderingen in *B. pertussis*.

Bevestiging van kinkhoest in het laboratorium wordt bemoeilijkt door de lage sensitiviteit van kweek, polymerase ketting reactie (PCR) en van het aantonen van een toename van antistoffen in gepaarde sera. Daarom, onderzochten we, of en op welk niveau, IgG antistoffen tegen pertussis toxine (IgG-PT) in een enkelvoudig serum monster indicatief zijn voor actuele of recente infectie met *B. pertussis* (**Hoofdstuk 9**). IgG-PT is gemeten in een ELISA in U per ml voor 7756 sera uit de algemene bevolking, in sera van 3491 patiënten met tenminste een viervoudige stijging in IgG-PT, in gepaarde sera van 89 patiënten met positieve kweek of PCR, en in sera van 57 patiënten met klinisch gedocumenteerde kinkhoest met een mediane follow-up duur van 1,4 jaar. Minder dan 1% van de deelnemers uit de studie in de algemene bevolking had IgG-PT niveau's van tenminste 100 U per ml. De specificiteit bij deze afkapwaarde voor positieve eenpuntsserologie bedroeg 99%. IgG-PT niveau's van tenminste 100 U per ml werden aangetoond in 80% van de sera van patiënten met klinische kinkhoest symptomen met tenminste een viervoudige titerstijging in IgG-PT in gepaarde sera. De follow-up sera van patiënten met klinisch gedocumenteerde kinkhoest, toonden aan dat binnen 1 jaar na het begin van kinkhoest symptomen bij meer dan 80% van de patiënten het IgG-PT niveau was gedaald tot minder dan 100 U per ml. In patiënten met positieve PCR of kweek bedroeg de sensitiviteit van een IgG-PT niveau van ten minste 100 U per ml 76%. Het aantal patiënten in de serodiagnostiek database met IgG-PT niveau's van tenminste 100 U per ml was meer dan vier keer groter dan het aantal patiënten met positieve tweepuntsserologie. Deze toename varieerde van een enkelvoudig stijging bij kinderen van 0 tot 5 maanden tot een veertienvoudige stijging bij personen van 15 jaar en ouder. We concludeerden dat IgG-PT niveau's van tenminste 100 U per ml een specifieke en sensitieve methode is om een recente of actuele infectie met *B. pertussis* te diagnosticeren. Diagnose op grond van een enkelvoudig serum monster kan het stellen van de diagnose kinkhoest bij oudere kinderen en volwassenen vereenvoudigen. Pas later in het beloop van de ziekte wordt bij hen laboratorium diagnostiek geïnitialiseerd. Daarom is deze methode niet alleen bruikbaar voor de individuele patiënt, maar ook voor epidemiologisch onderzoek.

In de algemene discussie (**Hoofdstuk 10**) zijn methodologische aspecten besproken van selectieve participatie, leeftijd versus cohort effect en de validiteit van vaccinatiestatus bij de interpretatie van de resultaten van de studies in de algemene bevolking. Voor de surveillance gegevens van kinkhoest is ingegaan op de validiteit van gegevens van aangiften en van schattingen van vaccin

effectiviteit op grond van surveillance gegevens. Voor de studie naar IgG-PT niveau's in een enkelvoudig serum monster als diagnostische methode bij kinkhoest, werden methodologische aspecten zoals de validiteit van IgG-PT niveau's van patiënten met een viervoudige titerstijging als karakteristiek voor reconvalescentie sera besproken.

Implicaties van de resultaten uit de diverse onderzoeken werden besproken in het licht van (verdere) beheersing van de ziekten. De difterie epidemie in de voormalige Sovjet Unie heeft laten zien dat deze ziekte kan terugkeren in vatbare populaties. Het gebrek aan difterie antitoxine antistoffen bij een groot deel van de volwassenen ten gevolge van afnemende immuniteit, geven aan dat het bestuderen van de (memory) respons van volwassenen zonder detecteerbare of met geringe antistof niveau's inzicht kan geven of na blootstelling aan *C. diphtheriae* vertrouwd kan worden op voldoende immuniteit.
Tetanus vaccinatie induceert zeer goede immuniteit. Dit betekent dat tetanus goed kan worden voorkomen door vaccinatie te richten op ongevaccineerden. Primaire vaccinatie van ongevaccineerde volwassenen geboren voor de introductie van het vaccinatieprogramma lijkt het meest effectief te zijn.
De jarenlange ervaring van Nederland met geïnactiveerd polio vaccin toont aan dat dit vaccin zeer goede bescherming en voldoende groepsimmuniteit geeft. Om vaccin-geassocieerde poliomyelitis te voorkomen kan het in het late stadium van de wereldwijde polio eradicatie zinvol zijn, over te gaan van oraal polio vaccin naar geïnactiveerd polio vaccin.
De mogelijkheden voor interventie in verband met de hiaten in de immuniteit tegen difterie, tetanus en poliomyelitis onder orthodox gereformeerde groepen zijn beperkt. Het bestuderen van circulatie van de pathogenen zal het opsporen van de ziekten wanneer zij terugkeren in een zo vroeg mogelijk stadium mogelijk maken.
Door invoering van een booster vaccinatie na de zuigelingen vaccinaties kan kinkhoest beter worden voorkomen. Aanpassing van zo'n vaccin op circulerende stammen zou effectief kunnen zijn. De keuzes zowel voor het meest geschikte vaccin als voor de leeftijd van boostering worden gecompliceerd door gebrek aan inzicht in de lange termijn effecten op de kinkhoest epidemiologie. Naast surveillance gegevens zijn ook gegevens over de reservoirs voor infecties met name voor ongevaccineerde zuigelingen van belang om het effect op de epidemiologie van kinkhoest te bestuderen.

Over het algemeen laten onze resultaten zien dat niet alleen de hoge vaccinatiegraad onder kinderen gehandhaafd moet worden maar dat vaccinatie ook gericht zou moeten worden op volwassenen. Aanpassing van vaccins op circulerende stammen zou effectief kunnen zijn voor andere vaccins dan kinkhoest en influenza.

Om veranderingen in epidemiologie van ziekten waartegen wordt gevaccineerd te blijven bestuderen, zouden epidemiologische studies zoals beschreven in dit proefschrift periodiek herhaald moeten worden om het succes van (nieuwe) vaccinatieprogramma's te verhogen.

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About the author

Hester Ellen de Melker was born on the 27th of July, 1968 in Winschoten, the Netherlands. She finished secondary school (Gymnasium-β at the Katholieke Scholengemeenschap "de Breul" in Zeist) in 1986. In 1987 she completed a secretarial course (Opleidingsinstituut Schoevers in Utrecht). In the same year she started her studies in Biomedical Sciences at the University of Leiden and graduated with a major in epidemiology in 1993. Subsequently, she works as epidemiologist at the Department for Infectious Diseases Epidemiology, National Institute of Public Health and the Environment in Bilthoven. Her research involves mainly research projects directed to vaccine-preventable diseases and evaluation of the national immunisation programme. The work for her Ph D was realised within these projects and included particularly the design of a serum bank of the general population for seroprevalence studies on vaccine-preventable diseases (*Pienter-project*) and pertussis surveillance in the Netherlands.
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