Outbreaks: Sources of Epidemiological Knowledge in Communicable Disease Control

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Colophon

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Outbreaks: Sources of Epidemiological Knowledge in Communicable Disease Control

Uitbraken: bronnen van epidemiologische kennis voor het beheersen van overdraagbare ziekten

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"Our aim is therefore to study outbreaks of communicable diseases as fully as possible in the confident expectation that, as in other branches of science, knowledge will bring enlightenment". (Vrij naar: Wilson G. The hazards of immunization. London: The Athlone Press, 1976)

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List of abbreviations

AIDS	Acquired Immuno-Deficiency Syndrome
BI	Betrouwbaarheidsinterval
C	Celsius
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GA	Georgia
GAVI	Global Alliance for Vaccines and Immunization
GGD	Gemeentelijke (gewestelijke) gezondheidsdienst
GI	Genogroup I
GMC	Geometrical mean concentration
GMP	Good manufacturing practice
H/h	Hour
HACCP	Hazard analysis and critical control points
HIV	Human Immunodeficiency Virus
IgA	Immunoglobulin A
IGB	Inspectie Gezondheidsbescherming, Inspectorate for Health Protection
IgG	Immunoglobulin G
IgG-PT	Immunoglobulin G pertussis toxine
IPV	Inactivated polio vaccin
IU/ml	International units per milililtre
KvW	Keuringsdienst van Waren, Food Inspection Service
LR	Likelyhood ratio
MPHS	Municipal public health service
MPR	Municipal population register
MRSA	Methicilline Resistente Staphylococcus Aureus
NaCl	Natriumchloride
NLV	Norwalk-like virus
OPV	Oral polio vaccin
OR	Odds ratio
P	Probability
PCR	Polymerase chain reaction
PHS	Public health service
RIVM	Rijksinstituut voor Volksgezondheid en Milieu, National Institute for Public Health and the Environment
RR	Relative risk
RT	Reverse transcription
SARS	Severe Acute Respiratory Syndrome
SAS	Statistical Software
SD	Standard deviation
SPSS	Statistical Package for Social Sciences
U/ml	Units per milililtre
WHO	World Health Organisation
X^2	Chi-kwadraat

General introduction

chapter

1

Communicable diseases and public health

Public health has been defined as the science and art of disease prevention, prolonging life, and promoting health and well-being through organized community effort for the sanitation of the environment, the control of communicable infections, the organization of medical and nursing services for the early diagnosis and prevention of disease, the education of the individual in personal health and the development of the social machinery to assure everyone a standard of living adequate for the maintenance or improvement of health.[1]

In the era of the eradication of smallpox in the sixties and seventies of the last century the world was optimistic about a future world with controlled communicable diseases. The eradication of smallpox inspired the world to eradicate poliomyelitis as well. This optimism was in vain. The poliomyelitis eradication experienced drawbacks, resulting in an all or nothing battle.[2] Also old infectious diseases like Influenza, Dengue and Tuberculosis have revived and Legionella, HIV and SARS and the development of antibiotic resistance in the form of MRSA and multi-drug resistance of *Mycobacterium tuberculosis* are new examples of emerging communicable diseases.[3]

Microbial agents that cause communicable diseases have proven to be very dynamic, resilient, and well adapted to exploit opportunities for change, development and emergence of new species and for spread. In the "global village" the increase of travelling of humans and animals and the enormous increase in meat industry and its antibiotic use, have created new opportunities for sexually transmitted diseases, zoonoses, antibiotic resistance and emerging diseases.

The yearly world wide mortality due to communicable diseases is 14 million death.[4] The major part of this mortality occurs in the developing world, where nearly half of all deaths are due to communicable diseases. Of this mortality, 90% is due to acute diarrhoeal and respiratory infections of children, AIDS, tuberculosis, malaria, and measles. In developing countries millions are also still suffering from disabling diseases, such as poliomyelitis, leprosy, lymphatic filariasis, and onchocerciasis. In many of these countries communicable diseases overwhelm the capacity of health systems.

In the developed world serious challenges to the health system are likely to occur as well when the next inevitable influenza pandemic occurs, or after the deliberate

release of pathogens by bio-terrorists, or when any other major outbreak occurs. China's public health system required major upgrading to counter a threat such as SARS.[5] The public health significance of communicable diseases in terms of human suffering, deaths, and disability, public fear and fear among health care workers for emerging diseases like Ebola and SARS, is aggravated by their negative consequences on economic growth and development.

The control of several communicable diseases has been problematic due to lack of effective vaccines, lack of therapeutic drugs and drugs becoming ineffective due to antimicrobial resistance. However, new initiatives such as the Global Fund to Fight AIDS, Tuberculosis and Malaria, and the Global Alliance for Vaccines and Immunization (GAVI) offer new perspectives for improvement.
Recently, the occurrence of emerging and re-emerging communicable diseases, and critics such as the journalist Laurie Garrett in her book 'Betrayal of Trust' have inspired countries to improve their public health infrastructures for communicable disease control. Laurie Garrett wrote: *"Were a naturally arising epidemic or a biological attack to occur, the public would only have one viable direction in which to place its trust: with its local, national, and global public health infrastructure"*.[6]

Motivation for this thesis

The first ideas for this thesis arose during the investigation of an outbreak of coughing in a primary school in 1986 in Heel, a village of around 3000 inhabitants in the province of Limburg.[7] The reason to study this outbreak was the concern of a general practitioner and a school physician about an epidemic of coughing of unknown cause, which had lasted several weeks already. The coughing children seriously disturbed the lessons and several of the children were exhausted due to nightly coughing and vomiting after cough attacks. The two doctors did not consider the diagnosis pertussis because of the high vaccination coverage of the children (99%). The type of coughing was, however, very similar to the typical pertussis coughing which can be observed in the tropics. A retrospective cohort study was therefore started of the population at risk, including the school children (n=147), the teaching staff (n=11), and household members (n=100) of children and teaching staff with and without pertussis, in order to reveal the cause of the disease and the extent of the epidemic. Structured questionnaires were used to study the extent of the epidemic and the nature of the complaints, and nasopharyngeal samples were taken for culture and blood samples for serological investigation.

At first the high serological antibody titres were interpreted as laboratory mistakes by the laboratory which executed the serological tests (the RIVM in Bilthoven). A mistake was ruled out with a repetition of all serological tests by the same laboratory. In the end, the outcome of the tests supported the hypothesis that it was indeed a pertussis epidemic in a group of well-vaccinated children.

The study did not only reveal the diagnosis pertussis (pertussis was confirmed by 10 positive cultures of *Bordetella pertussis*, the causative agent of pertussis), but also several other dimensions of knowledge concerning the agent, transmission and the host were obtained. This study revealed that a pertussis epidemic occurred among well-vaccinated primary school children and their teachers and families, and that a vaccination coverage of 99% did not provide sufficient herd immunity to stop transmission of *Bordetella pertussis* in this group. The secondary attack rate was significantly higher in families of teachers and school children with pertussis, compared to those without pertussis. The incidence of pertussis increased with age in the age group 6-13 years, probably because the immunity against pertussis decreased with increasing duration since the vaccination in the first year of life. The incidence of pertussis in children vaccinated with 16

Opacity Units per doses vaccine was higher than in children vaccinated with 10 Opacity Units.

At the level of diagnosis the sensitivity and specificity of single IgA and IgG antibody titres were evaluated in relation to the outcome of clinical symptoms. This led to the formulation of questions regarding the course and sensitivity and specificity of IgA and IgG antibodies for the diagnosis of pertussis.

The study was the first description in the Netherlands of the re-emergence of pertussis in a population with excellent vaccine coverage. It generated knowledge about pertussis at the level of determinants of pertussis and interventions against the disease. It was therefore advised to reintroduce pertussis vaccination at the age of four, which was established as of 1998.

The study was performed at the time of a flourishing discussion about the meaning of the increase of reported pertussis since 1980 by the national surveillance system in the Netherlands.

On the one hand, this increase was hypothesized to be a true increase of the incidence of pertussis. This true increase was attributed to "infection pressure" from neighbouring countries with a less successful national vaccination program or no program at all (England, Belgium, Germany, Sweden)[8,9] and to a lowering of the Opacity Units between 1976 and 1984 from 16 to 10 Units.[9]

On the other hand the increase of reported pertussis was hypothesized to be no true increase of the incidence of pertussis because the pertussis vaccine had remained effective in spite of lowering the Opacity Units between 1976 and 1984.[10] The increase of reported pertussis was attributed to the increased interest of doctors in pertussis because of the serious epidemic in England in the periods 1978-1979 and 1981-1982,[11] and the availability of a new diagnostic single serological IgA and IgG test for pertussis since 1981.[10-16]

In 1986-1987 the number of reported pertussis increased sharply.[17-20]

In 1987 it was hypothesized, based on the interpretation of surveillance data of reported pertussis, that the 1986-1987 increase was no true increase in the incidence of pertussis and was attributed to the introduction of the single IgA and IgG test[10] and lack of clinical, laboratory and epidemiological criteria for

the diagnosis pertussis. It was argued that due to the lack of such criteria not only "serious cases of pertussis" were reported, but also atypical cases.[21] This dominant hypothesis led to the decision in 1987 to change the sensitivity and application of the diagnostic criteria for pertussis, through defining strict clinical criteria for pertussis and requiring a paired instead of a single serological test for confirmation of pertussis infection.[21-24]

This decision was followed by a sharp decrease of the reported pertussis cases in 1988.[20]

This 1986 study[7] supported the hypothesis of a true increase in the pertussis incidence, which in the meantime has been accepted.[25,26] The study also rejected the 10-16 Opacity Unit hypothesis as an explanation for this true increase.

The experiences gained in this epidemiological study of a pertussis outbreak, together with those gained in studies of some other outbreaks,[27-29] suggested that communicable disease outbreaks can be seen as natural experiments. These experiments may not only generate specific knowledge for the containment of the outbreak as such, but may also generate more general scientific knowledge about determinants and interventions of a communicable disease. This idea is the central theme of this thesis, which attempts to illustrate the relevance of epidemiological studies of outbreaks of communicable diseases for gaining more general knowledge.

Determinants and interventions of communicable diseases

A communicable disease is a clinically manifest disease of humans or animals due to a specific infectious agent or its toxic products, that arises through transmission of that agent or its products from an infected person, animal or inanimate source to a susceptible host; either directly or indirectly through an intermediate plant or animal host, vector or the inanimate environment.[3]

The three basic determinants of communicable diseases are (1) the infectious agent and its sources, (2) transmission of the agent to the host and the environment in which transmission takes place, and (3) the host, or a group of hosts including the routes of entry into the host, and the reaction of a host or a group of hosts to the infection with an agent.[3,30,31]

Interventions to control communicable diseases control are directed towards these three determinants of communicable diseases:

- The elimination or containment of the sources of infectious agents or of the infectious agent as such. This is the field of environmental control such as provision of clean air, water, milk and food and the proper management of sewage and garbage.
- The interruption of the chain of transmission, such as the control of insects and vectors of disease, but also technical hygiene like masks and protecting clothing and avoiding contact between hosts and groups of hosts, (e.g. closing schools, avoiding crowding, quarantine and isolation). Also avoiding infection with micro organisms as in case of sexually transmitted diseases is considered as an interruption of the chain of transmission.
- The protection of the hosts against infection or disease or both. This can be reached through passive and active immunization (vaccination), antibiotic and antiviral prophylaxis and therapy.[31] The maintenance of good health of hosts and populations of hosts, undergoing first and natural re-infections can also be considered as a form of protecting hosts against communicable diseases.

Interventions in communicable disease control are divided as well in primary and secondary prevention at individual and population level. The most successful form of primary prevention is the national vaccination programme resulting in immunity at individual level and population level (herd immunity). In addition,

education programmes towards avoiding infection with sexually transmitted diseases like HIV are successful forms of primary prevention, especially in democratic countries with an open and taboo free atmosphere among parents, teachers and children for sexual education. Secondary prevention at individual level is given in the health care system and at population level in screening programmes and outbreak management.

Scientific knowledge and public health practice to control communicable diseases

Communicable disease control is based on both scientific knowledge about the determinants and interventions of communicable diseases and on public health practice.

Public health practice includes (1) the application of scientific knowledge of determinants and interventions in order to control communicable diseases, (2) surveillance in order to guard the control of communicable diseases through monitoring the occurrence of communicable diseases and through detecting outbreaks of communicable diseases, and (3) the management of outbreaks.

The application of scientific knowledge about determinants and interventions of communicable diseases may result in a successful control of communicable diseases. Surveillance is used to guard this success.

Surveillance

The term *surveillance,* derived from the French word meaning "to watch over," may be defined as a system of close observation of all aspects of the occurrence and distribution of a given disease through the systematic collection, tabulation, analysis, and dissemination of all relevant data pertaining to that disease. Although the methodology of surveillance is basically descriptive, its function has been considered as of far greater importance than merely collective and archival: "Surveillance must be dynamic, current, and is purposeful and fundamental to prompt and effective control and prevention of disease".[32]

In communicable disease control surveillance has been defined as the permanent process of systematic collection, orderly consolidation, analysis, interpretation and evaluation of pertinent data with prompt dissemination of the results and processed information to those who need to know, particularly those who are in a position to take action.[3,31]

There is a prolonged concern about the quality of surveillance in communicable disease control. Traditional methods of surveillance in communicable disease control are based on *cases* of clinical disease, reported by physicians or identified by some survey technique. The adequacy of surveillance based on such clinical cases requires the occurrence of clinical illness, sufficient severity to seek medi-

cal care, availability of medical care, capability of physicians to diagnose illness, laboratory support of diagnosis, reporting of disease and collection and analysis of data by health departments.

In many countries, however, reported cases of communicable diseases still form the backbone of input of their national surveillance system for communicable disease control. Therefore, high-quality data are needed if surveillance and assessment information are to be relied upon in public health decision-making. This implies that attention must be given to the quality of data in designing, implementing, and evaluating surveillance systems and analytic and evaluative studies. Analysis and interpretation of surveillance data are only as good as the quality of the data collected, (the "*garbage in, garbage out*" principle).[33] Following this understanding, attention has been given to improve reporting in communicable disease surveillance. This included the motivation of reporters other than those related to disease reporting laws, by emphasizing, for example, professional gain and by summarizing surveillance data, improving the ease of reporting and defining specific case definitions for accurate reporting. Active reporting was added to the original passive reporting. In active surveillance, the reporter is contacted at regular intervals and specifically asked about the occurrence of the disease(s) under surveillance. Thus, there is an active attempt by public health officials to obtain disease occurrence information from the reporter. Also reporting by sentinel physicians, laboratory reporting and serological surveillance were introduced to compensate for the drawbacks of surveillance.[32]

In the past decades concern about communicable disease surveillance has been growing internationally, especially about surveillance as a method to obtain valid data.[34-40] Therefore it was suggested that data sources and surveillance methods must be carefully selected to match the specified goals of surveillance and to maximize the attributes (timeliness, sensitivity, positive predictive value, simplicity, flexibility) of greatest importance at each level of the public health system for each health event or determinant.[41] However the requirements for surveillance to obtain valid data may be missing or inadequate, and even in highly developed countries the reporting of communicable diseases is less than satisfactory and involves much variability.[42]

Core activities of communicable disease surveillance systems remain collection, analysis, and dissemination of information about health events under surveillance. Doing these well requires attention to the mechanics of surveillance,

such as making the health department accessible at all times to receive reports from the health care system and provide consultation, and maintaining current directories of persons for dissemination of surveillance data, alerts, and recommendations. Although rapid access to electronic representations of health events (e.g., laboratory reports, patient records, or health care claims) may provide great opportunities for more timely and complete surveillance, some important information (e.g., exposures, contacts) will still need to be collected directly from affected persons, through the health care system.[43] So even this modern technology may not be able to compensate for the basic drawbacks of communicable disease surveillance, because principally modern information technology remains dependant on the quality of reporting in the health service systems.

In modern times determinants of the outcome of surveillance in communicable disease remain multi-factorial and therefore surveillance data might be used to monitor health events and determinants, and to formulate hypotheses, but not to test hypotheses. So surveillance should lead to, but not be confused with, research.[33] Indeed, there is a still increasing realisation that the quality improvement of surveillance in communicable disease control due to modern information technology is less in providing knowledge for control of communicable disease and adequate information for containment of individual outbreaks and more in early warning of cases of infectious diseases, outbreaks, and emerging and re-emerging diseases.[44-46]

Outbreak management

Unsuccessful control of communicable diseases and re-emerging and emerging of these diseases may result in outbreaks of communicable diseases in a host or in a group of hosts. An outbreak or epidemic is the occurrence, in a defined community or region, of cases of an illness with a frequency clearly in excess of normal expectancy, the background incidence. Epidemicity is thus relative to usual frequency of a disease in a specified population in a specified area in a specified time and does not depend on the absolute number of cases. A single case of a new communicable disease in a population can be considered as an epidemic and two new cases as evidence of transmission. Endemicity refers to a habitual presence of a disease or infectious agent within a given geographic area or a population group. It may also refer to the usual prevalence of a given disease within such an area. Hyperendemicity expresses a habitual presence at all ages at a high level of incidence.[3]

The steps in surveillance to detect outbreaks are (1) detecting signals of a communicable disease outbreak, and (2) combining signals to suspect an outbreak. Outbreaks of communicable diseases may require outbreak management. Important to outbreak management is a coordinated investigation and response involving health workers including clinicians, epidemiologists, microbiologists, health educators and the public health authorities. The best way to ensure coordination is usually to establish an outbreak containment committee early in the outbreak. The goal of outbreak management is containment of the specific outbreak through the principles of communicable disease control: interruption of the transmission of the infectious agent and management of infected hosts.[3,31,47]

In order to establish an etiological diagnosis and to reach the goal of containment of an outbreak, the possible steps in an outbreak response should be systematic and based on epidemiological evidence, despite the fact that public and political reaction, urgency and the local situation may make this difficult.[3,31,47]

Epidemiology and proof of causation

In communicable disease control, epidemiology is used to obtain evidence about determinants and interventions.

Epidemiology is the study of distribution of health and disease in populations and of its determinants.[48] Regarding communicable diseases, it is a quantitative science concerned with the circumstances under which infectious disease processes occur, the factors that affect their incidence and spread, and the use of this knowledge for prevention and control.[49]

Serological epidemiology is the systematic testing of blood samples from a defined sample of a population for the presence of antibodies, antigens, genetic markers, specific cell-mediated immunity, and other biological characteristics. It constitutes an important epidemiological tool. Serological techniques can (1) identify the past and current *prevalence* of an infectious agent in a community; (2) identify the *incidence* of infection by sero-conversion or a rise in titre in samples obtained at two different times; (3) reveal the ratio of sub-clinical to clinical infections, when combined with clinical data; and (4) estimate the need for immunization programmes and estimate their effectiveness as to the presence, level, and quality of antibody produced; its duration; and the degree of protection against disease.

New developments in the study of communicable disease control are molecular epidemiology and mathematical modelling of transmission dynamics of infectious diseases.

Epidemiological methods can be divided into descriptive, analytical and experimental.[31] Epidemiological methods are used to compare the effectiveness of new antibiotics or of new therapies in treatment or prophylaxis, to delineate the etiological importance of a new micro-organism, to establish normal laboratory values, to assess risk factors that affect infection, disease, and prognosis, and in many other clinical and laboratory settings.

With descriptive epidemiology, the circumstances under which infection and disease occur in a population are described. This includes data on prevalence and incidence, epidemic behaviour, and the spatial and temporal distribution of infection and disease, as well as the characteristics of the host such as age, sex, race, occupation, familial or other setting, socio-economic level, nutritional status, and genetic background. Analytical epidemiology is a pre-planned manner to weigh the importance of factors involved in infection or disease and to evaluate a hypothesis of causation, or to measure the effectiveness of an immunization or control programme. The major analytic methods used are the retro- and prospective cohort study and the retrospective case-control and cross-sectional study. Most efforts of this type represent special studies in specific population groups. Experimental epidemiology represents planned experiments designed to control the influence of extraneous factors, among those exposed or not exposed to an etiological factor, preventive measure, or environmental manipulation by the investigator. An example is the planned introduction of vaccine or infectious agent in a controlled fashion into a population of volunteers or animals and the analysis of the spread of infection and disease within these groups as compared to a non-exposed group. Such studies offer the most scientifically controlled method of epidemiological study. Natural experimental studies make use of existing natural circumstances, which give the opportunity to study clues for infectious disease control.

The information obtained from epidemiological studies can be utilised in several ways. It may help elucidate the aetiology of a specific disease or group of diseases by combining epidemiological data with information from other disciplines such as genetics, biochemistry and microbiology. The information can also be used to evaluate the consistency of epidemiological data with etiological hypotheses

developed either clinically or through (natural) experiments. Finally, it may provide the basis for developing and evaluating preventive procedures and public health practices.

Evidence for a causal relationship between a factor and a disease is provided in the most direct way through experimentation and through the determination of biological mechanisms. Epidemiological studies usually provide very strong support for hypotheses of either a causal or indirect association. However, inferences from such studies are not made in isolation; they must take into account all relevant biological information. Epidemiology and other evidence can accumulate to the point where a causal hypothesis becomes highly probable. Unfortunately, it is not yet possible to quantify the degree of probability achieved by all the evidence for a specific hypothesis about the cause of a disease, so an element of subjectivity remains. Nevertheless, a causal hypothesis can be sufficiently probable to provide a reasonable basis for preventive and public health action.

Following the principles of proving causation,[50-52] a unified concept of causation that parallels the Henle Koch postulates has been developed, which is generally applicable to both communicable and non-communicable diseases:[53]

- Prevalence of the disease should be significantly higher in those exposed to the hypothesized cause than in controls not so exposed, because the cause may be present in the external environment or as a defect in host responses.
- When all other risk factors are held constant, exposure to the hypothesized cause should be more frequent among those with the disease than in controls without the disease.
- Incidence of the disease should be significantly higher in those exposed to the cause than in those not so exposed, as shown by prospective studies.
- Temporally, the disease should follow exposure to the hypothesized causative agent with a distribution of incubation periods on a log-normal-shaped curve.
- A spectrum of host responses should follow exposure to the hypothesized agent along a logical biologic gradient from mild to severe.
- A measurable host response following exposure to the hypothesized cause should have a high probability of appearing in those lacking this before exposure or should increase in magnitude if present before exposure; this response pattern should occur infrequently in persons not so exposed.
- Experimental reproduction of the disease should occur more frequently in animals or man appropriately exposed to the hypothesized cause than in those

not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the laboratory, or demonstrated in a controlled regulation of natural exposure, the natural experiment.

- Elimination or modification of the hypothesized cause or of the vector carrying it should decrease the incidence of the disease (e.g., control of polluted water, removal of tar from cigarettes).
- Prevention or modification of the host's response on exposure to the hypothesized cause should decrease or eliminate the disease (e.g., immunization, drugs to lower cholesterol, specific lymphocyte transfer factor in cancer).
- All of the relationships and findings should make biologic and epidemiological sense.

A systematic approach of outbreaks for generating knowledge

In order to reach the goal of containment of an outbreak, I suggest 22 steps, which sometimes are taken concurrently, as shown in Table 1.[3,31,47] My argument for this systematic procedure is that it integrates surveillance, epidemiological methods and outbreak management. This systematic and integrative approach creates the opportunity to generate knowledge to contain the outbreak of the communicable disease at hand, and to generate knowledge of a more general nature, included the testing of hypotheses generated through surveillance, about the communicable disease.

Table 1. Steps to contain an outbreak.

A. Confirmation of an outbreak and establishment of its aetiology

1 Verify the diagnosis
2 Confirm the existence of an outbreak through comparing with previous surveillance data on the disease
3 Establish an etiological diagnosis if possible and go to C
4 If an etiological diagnosis cannot be established, go to B

B. Epidemiological investigation of an outbreak

5 Define the condition epidemiologically and clinically
6 Collect material for isolation and culture and for serological tests
7 Investigate the extent of the outbreak by a quick survey of hospitals, physicians and other sources, in order to identify the infected and possible affected persons and their characteristics
8 Record case histories and if possible identify additional cases
9 Investigate the basic epidemiological characteristics in terms of time, place and person (age, sex, occupation, ethnic groups), possible routes of transmission, methods of spread and ports of entry, and the spectrum of clinical illness
10 Prepare, if necessary, a spot map of cases
11 Draw an epidemic curve
12 Seek a common denominator and define the population at risk
13 Formulate a hypothesis about the nature of the agent causing the outbreak, its source, its way of transmission and its port of entry, and possible ways of control, and about the susceptibility of the host, or the groups of hosts, and the population at risk
14 Test the hypothesis by determining infections and illness rates in persons exposed and not exposed to putative source(s) by questionnaires, interviews, and laboratory tests. Try to isolate the agent from the putative sources(s)
15 Extend epidemiological and laboratory studies to other possible cases or to persons exposed, but not ill
16 Analyse the data and consider possible interpretations
17 On the basis of the analysis formulate short and long term control measures

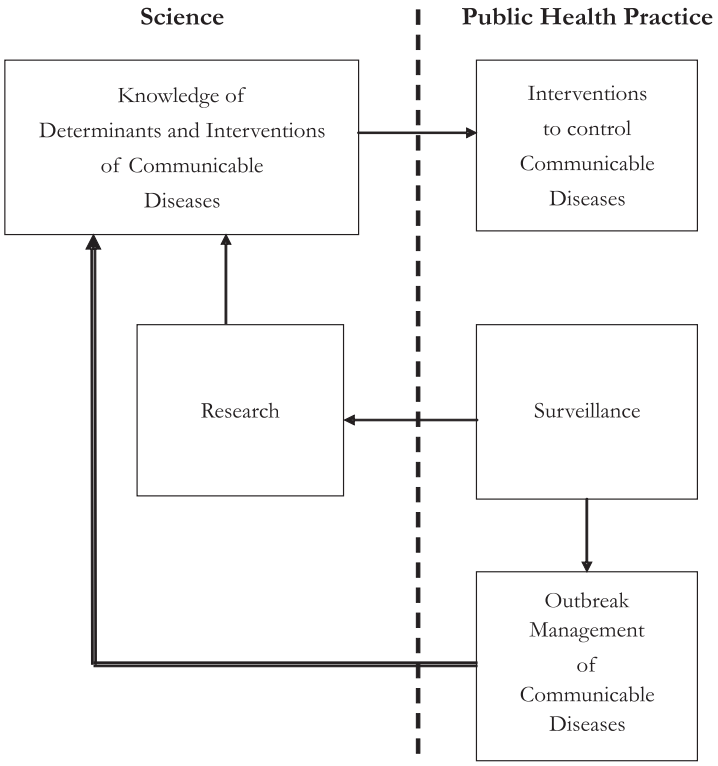
C. Containment of the outbreak

18 Contain the outbreak through control measures to prevent transmission and through protection of infected hosts against infection
19 Consider conducting ongoing disease surveillance in the acute phase of an outbreak in order to control the outbreak initially. This can be reached through keeping persons at risk (e.g. contacts of infectious hosts, the sources) under surveillance for disease onset. This form of surveillance is known as "source and contact tracing"
20 Consider continuation of community surveillance after the outbreak has initially been controlled, in order to identify additional cases and to complete containment
21 Prepare interval reports during and after the containment, in order to inform the general public and the public health authorities about the nature of the outbreak and what is required to contain the outbreak

D. Publish the outbreak management

22 Prepare a scientific report of the management of the outbreak for publication in a medical journal or epidemiological bulletin, in order to learn from the way the outbreak was managed and if applicable to present knowledge of more general nature about the communicable disease at hand

Figure 2. A model of communicable disease control.



A model of communicable disease control

In the previous paragraphs we have introduced the three basic determinants of communicable diseases (infectious agent, transmission and host) and explained how knowledge about these determinants will lead to the development of successful preventive interventions. Research of the determinants of communicable diseases and the development of interventions is the domain of science. The implementation of interventions (control) is in the domain of public health practice. Unsuccessful control may lead to outbreaks of communicable diseases. These outbreaks are usually detected through surveillance systems. We established the need for an etiologic diagnosis to reach the goal of containment of an outbreak, argued that epidemiological evidence is necessary, and described the systematic steps in an outbreak response to be followed to reach this goal. Finally, we introduced surveillance as a tool in the control of communicable diseases. We stated that surveillance data may be used to monitor health events and determinants, and to formulate hypotheses, but not to test hypotheses. So surveillance should lead to, but not be confused with research. Figure 2 depicts

the various aspects of communicable disease control and how the domains of science and public health practice interrelate.

Research questions

This thesis focuses on outbreak management, the domain of public health practice, as an opportunity for obtaining knowledge, basically the domain of science, in communicable disease control.

The research questions are:

1. Are epidemiological studies during outbreak management able to generate specific knowledge for the containment of the communicable disease at hand?
2. Are epidemiological studies during outbreaks management able to generate knowledge of a more general nature than necessary for the immediate containment of the communicable disease at hand?

Outline in this thesis

In **chapter 2 to 8** we give examples of epidemiological studies of communicable disease outbreaks and of studies arising from these outbreak studies. In the studies, knowledge is generated for communicable disease control.
Chapter 2 describes a study of an epidemic of pertussis among elderly people in a religious institution in the Netherlands. **Chapter 3** describes a study about the age-specific long-term course of IgG antibodies to pertussis toxin after symptomatic infection with *Bordetella pertussis*. **Chapter 4** describes the sensitivity and specificity of single IgA and IgG antibodies for early diagnosis of pertussis in adults as tools for outbreak management in public health practice. **Chapter 5** describes a study in which the pertussis outbreak, described in Chapter 2, is associated with social isolation among elderly nuns in the convent.
Chapter 6 describes an epidemic of *Salmonella typhimurium* associated with traditional salted, smoked, and dried ham. **Chapter 7** describes a gastroenteritis epidemic caused by a Norwalk-like virus after two weddings in a restaurant and provides a plea for integral microbiological investigation.
Chapter 8 describes the risk of introduction of poliovirus into a Cape Verdian community in the Netherlands during an outbreak of poliovirus in Cape Verde in 2000.
Finally, in **chapter 9**, **the general discussion**, the research questions are answered and recommendations are provided.
The work reported on in this thesis is **summarized in chapter 10**.

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Part 1. Pertussis

chapter

2

An epidemic of pertussis among elderly people in a religious institution in The Netherlands

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Abstract

An epidemic of pertussis is described among elderly people in a religious institution in the Netherlands in 1992.

Subjects were evaluated for their vaccination status and for history and presence of respiratory symptoms. Specimens were collected for culture, polymerase chain reaction, and serological evaluation.

None of the 75 residents and 19 of 24 non-resident personnel had been vaccinated against pertussis. The overall attack rate of clinical pertussis, defined as persistent cough lasting at least 2 weeks, was 49%. In five subjects with clinical pertussis, either culture or polymerase chain reaction or both were positive for *Bordetella pertussis*. A significant (at least 4-fold) change in specific antibody titre was observed in 85% (41/48) and 20% (10/49) of subjects with and without clinical pertussis, respectively ($P < 0.0001$, chi-square 41.1). The attack rate of laboratory-confirmed pertussis was 42% (41/98). This rate was 5% (1/19), 20% (1/5), and 53% (39/74) in vaccinated personnel, unvaccinated personnel, and unvaccinated residents, respectively (not significant). Among residents aged between 55–74 years and 75–94 years, the attack rates were 47% (17/36) and 58% (22/38), respectively (relative risk = 0.8; 95% confidence interval 0.5–1.3). Four of 75 residents (5%) died from intracranial bleeding, while they were symptomatic for pertussis.

It is concluded that the attack rate of pertussis was high among unvaccinated elderly and that pertussis tended to increase with age. There may be a considerable risk of mortality from pertussis in this population. Physicians should be alert to the diagnosis of pertussis in the elderly with nocturnal and prolonged periods of coughing.

Introduction

Bordetella pertussis is an important pathogen of the upper respiratory tract particularly in children. Worldwide, the pathogen represents a significant part of childhood morbidity, characterized by a persistent vigorous paroxysmal cough. This entity is recognized as whooping cough. Especially in developing countries, the disease is a major threat to health in childhood, complicated by wasting and brain damage.
Most developed countries vaccinate to prevent pertussis. In the Netherlands, 96% of the children complete the vaccination program 3, 4, 5, and 11 months after birth. Because the national vaccination program in the Netherlands was introduced in 1952, subjects born before that year have not been vaccinated.
In spite of the high degree of vaccination, whooping cough is reported at a rate of 1–4.2 cases per 100,000 people each year.[1] These rates are maximal for non- or incompletely vaccinated children at 1–4 years of age, amounting to 95.3 cases per 100,000 per year during the period 1989–1994. During that period, reports of cases in adults over 20 years of age were rare.[2] However, there is increasing evidence that adults are at risk of pertussis and play a role in transmission of the disease in the community.[2–8]

In this report, we describe an epidemic of pertussis among unvaccinated elderly and partly vaccinated personnel from a religious community in the Netherlands. We studied the symptoms, duration, and spread of the disease, and calculated demographic and clinical scores. From nasopharyngeal swabs, a routine culture for *Bordetella pertussis* and a polymerase chain reaction (PCR) designed to detect *Bordetella pertussis* was performed.
In addition, the changes of immunoglobulin (Ig)A and IgG class-specific antibody titres were determined in two or three consecutive samples.

Materials and methods

Subjects and procedures

All 99 subjects who resided or were employed in the convent, i.e. 75 resident nuns and 24 non-resident personnel, were evaluated for the presence of persistent coughing. All subjects were informed about the purpose of the study and all consented to participate.
Due to late reporting, the study started in week 9 of the epidemic. At that time trained interviewers, assisted by a nurse from the institution, collected information

about clinical manifestations suggestive of pertussis while blind to laboratory data. They asked retrospectively about the presence of prolonged illness with paroxysmal productive cough, data of disease onset, cough at night, inspiratory wheezing, whooping, and post-tussive emesis.[9] In addition, the emergence and duration of the same clinical manifestations were prospectively monitored; first on a daily basis and, later (when new cases no longer emerged), weekly until symptoms had subsided in all subjects. They asked for the subject's vaccination status and for a history of prior diseases, with particular emphasis on respiratory symptoms.

Laboratory evaluation

From each individual, two nasopharyngeal swabs were obtained at weeks 9 and 13 of the epidemic.

For each individual, one nasopharyngeal swab was immediately placed in transport medium containing charcoal agar, Oxoid CM119 agar (Oxoid, Unipath, The Netherlands), sodium-citrate-diluted sheep blood, cephalexin 40 mg/l (Oxoid, Unipath), and amphotericin B (Bristol-Myers Squibb BV, The Netherlands). Within 12 h after collection, the specimens were transported to the microbiology laboratory of the hospital at Heerlen, The Netherlands. Subsequently, specimens were inoculated immediately on prewarmed Bordet-Gengou agar plates (Oxoid CM 267, Unipath), supplemented with defibrinated sheep blood (12%) and amphotericin B (1.7 mg/ml). After inoculation, the agar plates were incubated in 5% CO_2 at saturation and at 37 °C for 7 days. The plates were evaluated daily for the presence of colonies with typical morphological characteristics. Isolated strains were identified as *Bordetella pertussis* if they passed the criteria for gram-negative, oxidase- and catalase-positive microorganisms, and if they specifically reacted with the antiserum against *Bordetella pertussis* (Wellcome 2.M.10, supplied by the National Institute for Public Health and the Environment, Bilthoven, The Netherlands).

For each individual, another dry swab was immediately sent to the National Institute for PCR analysis. The procedure of sample processing, PCR, and the analysis of PCR products were performed as described previously.[10] The primer recognition of repeated DNA elements specific for *Bordetella pertussis* and *Bordetella parapertussis* has also been described previously.[11]

In addition, at weeks 9, 13, and 60 of the epidemic, serum samples were obtained to evaluate the presence of antibodies against *Bordetella pertussis* and other respiratory pathogens. For measurement of *Bordetella pertussis* antibodies, enzyme

immunoassay procedures were used according to described methods.[12,13] For IgG-class antibody detection, purified pertussis toxin was used. For IgA-class antibody detection, a crude cell-wall preparation of *Bordetella pertussis* was used.[13] Antibody-binding activities were quantitatively expressed as units per millilitre as described elsewhere.[12,13]
In addition, the antibody response to influenza A and B viruses, parainfluenza 1, 2, and 3 viruses, respiratory syncytial virus, adenovirus, *Chlamydia psittaci*, *Mycoplasma pneumoniae*, and *Coxiella burnetii* were evaluated by routine enzyme immunoassays (National Institute for Public Health and the Environment).

The presence of active pulmonary tuberculosis was evaluated by means of a chest radiograph. In addition, repetitive sputum samples were evaluated for the presence of *Mycobacterium tuberculosis* by means of routine acid-fast staining and culture in the hospital at Heerlen, The Netherlands.

Definitions

Clinical pertussis was defined as the presence of a persistent cough for at least 14 days, which started during the epidemic of coughing.[9] Laboratory-confirmed pertussis was defined as clinical pertussis, combined with one of the following laboratory findings: (i) a *Bordetella pertussis* strain isolated from the nasopharynx; (ii) a reactive PCR or; (iii) a significant (at least 4-fold) increase or decrease of IgA and/or IgG antibody titres among serum samples obtained at weeks 9, 13, and 60 of the epidemic.

Statistical analysis

Clinical and laboratory data were collected in a database management system. For the statistical analyses, the Statistical Package for Social Sciences (SPSS, USA) was used. The analyses of frequency tables were performed with Pearson's chi-square test and by comparing risks.[14]

Results

Study population

The study was performed in a religious community (n = 99) consisting of 75 residents (all nuns) and 24 non-resident personnel (3 males, 21 females) with a mean age of 75 years (range 55–94 years) and 27 years (range 21–46 years), respectively. They all (100%) participated in the study. The residents and staff

members participated in daily common social and religious activities and shared meals in the dining room. Residents with severe complaints of upper respiratory tract infections were nursed in a separate care room in the institution.

Clinical characteristics.

In retrospect, the first case, a resident nun, experienced her onset of disease on 3 June 1992, designated as week 1. Overall, the crude attack rate of cough was 52% during the epidemic (52/99). The epidemic reached its peak at week 6 (median at day 34), as expressed in an epidemic curve (Figure 1). The last patient started coughing in week 12 of the epidemic. Coughing persisted for a mean period of 69 days (range 7–268 days). Retrospective evaluation of the epidemic's first 9-week period revealed 46 cases of recent or actual coughing illness. All but two of these 46 cases (95.7%) met the criteria for clinical pertussis. Prospective evaluation revealed six cases of cough. The overall attack rate of clinical pertussis was 49% (49/99).

Among residents and personnel, the distribution of cases showing symptoms of cough and clinical pertussis was assessed according to vaccination status and age category (Table 1). Among personnel and residents, the rate of clinical pertussis was 12% (3/24) and 61% (46/75), respectively. Among vaccinated and unvac-

Table 1. Morbidity, mortality and laboratory results by status of vaccination and age (years) in the study population.

Variables	All participants	Personnel		
	age 21-94 (n=99)	All age 21-46 (n=24)	Vaccinated age 21-43 (n=19)	Non-vaccinated age 21-46 (n=5)
Subjects with a cough	52/99	5/24	2/19	3/5
Subjects with clinical pertussis	49/99	3/24	1/19	2/5
Subjects with isolation of *Bordetella pertussis* or reactive PCR	5/99	1/24	1/19	0/5
Subjects with significant change in antibody titres	51/97[a]	4/24	3/19	1/5
Laboratory confirmed pertussis	41/98[b]	2/24	1/19	1/5
Mortality	6/99[c]	0/24	0/19	0/5
Pertussis-associated mortality	4/99	0/24	0/19	0/5

[a] From one subject with and one without clinical pertussis only one serum sample was obtained

[b] The subject without clinical pertussis from whom only one serum sample was obtained is added in the denominator

[c] The subject with clinical pertussis from whom only one serum sample was obtained is added in the denominator

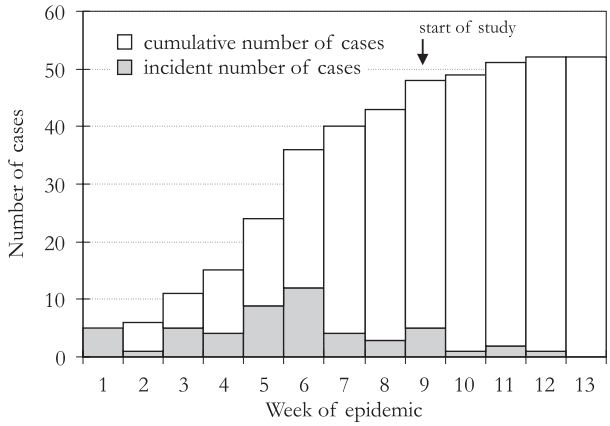

Figure 1. The epidemic curve of cases with persistent cough among 99 subjects in a closed religious institution. The first case was reported on 1 June 1992 (week 1) and the study was started at week 9 as indicated by an arrow. Grey bars present the cumulative case reports of clinical pertussis, the closed bars present newly reported cases of clinical pertussis. The first case was reported to the physician of the institution.

cinated personnel, the rate was 1/19 (5%) and 2/5 (40%), respectively [relative risk (RR) = 0.8, 95% confidence interval (CI) 0.3–1.5]. The rate was 59% (22/37) and 66% (25/38) for the unvaccinated residents from the age categories 55–74 years and 75–94 years, respectively (RR = 0.9, 95% CI 0.7–1.3)

Residents (non-vaccinated)		
All age 55-94 (n=75)	< 75 years age 55-74 (n=37)	≥ 75 years age 75-94 (n=38)
47/75	22/37	25/38
46/75	22/37	24/38
4/75	3/37	1/38
47/73	20/36	27/37
39/74	17/36	22/38
6/75	1/37	5/38
4/75	1/37	3/38

Laboratory evaluation

At weeks 9 and 13 of the epidemic, respectively, from 93 and 96 subjects a nasopharyngeal specimen was obtained for culture of *Bordetella pertussis*, and from 91 and 95 subjects a nasopharyngeal specimen was obtained for PCR. *Bordetella pertussis* was isolated in four of 93 (4%) subjects and PCR was reactive in two of 91 (2%) subjects. One case revealed both isolation of *Bordetella pertussis* and a reactive PCR. *Bordetella pertussis* isolates and reactive PCR results were only found in samples obtained during week 9 from subjects who met the criteria for clinical pertussis.

At weeks 9, 13, and 60 of the epidemic, 94, 97, and 85 serum samples were obtained for measurement of IgA and IgG antibody titres, respectively. At these times, the mean levels of IgA titres were 304, 313, and 124 U/ml in subjects with clinical pertussis, compared with 39, 36, and 19 U/ml, respectively, in the group of subjects that did not meet the criteria of clinical pertussis ($P < 0.0001$). Corresponding IgG titres were 1012, 743, and 82 U/ml in subjects who met the criteria for clinical pertussis, compared with 49, 30, and 15 U/ml, respectively, in subjects who did not meet the criteria of clinical pertussis ($P < 0.005$).

In order to detect significant changes between IgG and IgA antibody titres, three consecutive serum samples were collected from 80 subjects at weeks 9, 13, and 60 of the epidemic.
Two serum samples were obtained from 17 subjects.
From one resident with and from one resident without clinical pertussis, only one single serum sample was obtained. Therefore, these two subjects were not further evaluated for changes in antibody titres.
The 97 subjects from whom at least two serum samples were obtained were further evaluated for changes in antibody titres. Four (4%) subjects showed a significant (at least 4-fold) increase in IgA or IgG antibody titres. In seven (7%) subjects, both a significant increase and decrease in antibody titres were observed. Forty (40%) subjects showed a significant (at least 4-fold) decrease in antibody titres. Forty-six (47%) subjects showed no significant change in IgG or IgA antibody titres.

Forty-eight of 97 subjects from whom at least two serum samples could be obtained met the criteria for clinical pertussis; 49 subjects did not. Forty-one of 48 (85%) subjects with clinical pertussis and ten of 49 (20%) subjects without clinical pertussis showed a significant change in antibody titres against *Bordetella pertussis* ($P < 0.0001$, chi-square = 41.1).

Two of five subjects with a positive culture of *Bordetella pertussis* or a reactive PCR showed a significant increase of antibody titres. Three of these five subjects consecutively showed both a significant increase and a decrease of antibody titres.

The attack rate of laboratory-confirmed pertussis could be evaluated in 98 subjects, consisting of the 97 subjects described above and the subject without clinical pertussis from whom only one serum sample could be obtained. The attack rate of laboratory-confirmed pertussis was 42% (41/98) (Table 1). Among

personnel and residents, this rate was 8% (2/24) and 53% (39/74), respectively. Among the (unvaccinated) residents, the attack rate of laboratory-confirmed pertussis was 47% (17/36) and 58% (22/38) for the categories 55–74 years and 75–94 years of age, respectively (RR = 0.8, 95% CI 0.5–1.3). Among vaccinated and unvaccinated personnel, this rate was 5% (1/19) and 20% (1/5), respectively (RR = 3.8, 95% CI 0.3–50.7).

As determined by enzyme immunoassay, no serological evidence was present for infection with influenza A and viruses, parainfluenza 1, 2, and 3 viruses, respiratory syncytial virus, adenovirus, *Chlamydia psittaci*, *Mycoplasma pneumoniae*, or *Coxiella burnetii*.
The presence of pulmonary tuberculosis was excluded in ten symptomatic residents with the greatest clinical suspicion of tuberculosis.

Severity of laboratory-confirmed pertussis

Among personnel, laboratory-confirmed pertussis was observed in only two subjects and it did not run a severe course. They coughed for a period of 16

Table 2. Clinical variables of non-vaccinated elderly residents with laboratory-confirmed pertussis by age.

Variables	Age group		
	55-74 years (n=17)	75-94 years (n=22)	All (n=39)
Duration of cough (days)[a]			
- mean	65	67	66
- median	45	57	52
- range	14-268	14-159	14-268
- standard deviation	62	41	50
Duration of cough (days)[b]			
- 14-20	3	2	5 (13%)
- 21-41	5	4	9 (23%)
- 42-55	1	5	6 (15%)
- 56-69	4	3	7 (18%)
≥ 70	4	8	12 (31%)
Symptoms[b]			
- nightly cough	10	14	24 (61%)
- productive cough	2	1	3 (8%)
- inspiratory cough	6	3	9 (23%)
- whooping	5	2	7 (18%)
- posttussive emesis	2	3	5 (13%0

[a] Results given in days
[b] Results given in number of patients

days and 108 days, and accompanying clinical symptoms were not typical for pertussis. In contrast, among the residents with laboratory-confirmed pertussis, the median duration of cough was 55 days with typical accompanying symptoms (Table 2). For example, the prevalence of nightly cough was 61% (24/39) in this group and in 64% of the cases the cough lasted for more than 6 weeks. The prevalence of coughing lasting more than 6 weeks was 53% (9/17) and 73% (16/22) for the categories 55–74 years and 75–94 years of age, respectively (RR = 0.7, 95% CI 0.4–1.2).

Mortality

The death rate during the study period was 6% (6/99).

Two deaths were not associated with pertussis. One subject died of pancreatic carcinoma without signs of clinical pertussis. At weeks 9 and 13 of the epidemic, the IgA antibody titres of this subject were 55 U/ml and 29 U/ml, respectively. The corresponding IgG antibody titres were 56 U/ml and 46 U/ml. A second subject died of breast carcinoma without signs of clinical pertussis with an IgA antibody titre of 356 U/ml and an IgG titre of 224 U/ml at week 9.

The third and fourth subjects died after developing intracranial bleeding during the symptomatic episode of laboratory-confirmed pertussis. At that time, they had coughed for a period of 108 days and 159 days.

The fifth subject also died as a result of intracranial bleeding, after she had been symptomatic for clinical pertussis for 173 days. For this subject, the IgA antibody titre was 41 U/ml at week 13. The corresponding IgG antibody titre was 800 U/ml, which was significantly higher than the mean IgG titre of 30 U/ml for subjects without clinical pertussis at week 13.

The intracranial bleedings were diagnosed clinically by an experienced physician and based on the acute development of dysarthria, aphasia, and hemiplegia. The sixth subject died after a period of cough lasting 236 days, with IgA titres of 656 U/ml and 1120 U/ml and IgG titres of 8000 U/ml and 5824 U/ml at weeks 9 and 13, respectively. The antibody titres of this subject were significantly higher than the mean IgA and IgG titres of subjects who did not meet the criteria for clinical pertussis (mean IgA titres were 39 U/ml and 36 U/ml and mean IgG titres were 49 U/ml and 30 U/ml at weeks 9 and 13, respectively).

Presuming that the deaths of cases 3 to 6 were associated with the presence of pertussis, the pertussis-associated death rate was 5% (4/75) among residents.

Yield of laboratory tests to confirm clinical pertussis.
Early nasopharyngeal swabs and convalescent blood samples were not obtained from the majority of subjects. As a result, the yield of a positive test to confirm clinical pertussis was low, e.g. the yields were 4%, 8%, and 16% for a reactive PCR, a positive culture, and a significantly increasing antibody titre, respectively. In addition, among the subjects with clinical pertussis, concordant results of reactive PCR and positive culture were observed in only 20% of the cases. Concordant results between positive culture and a significant increase in antibody titre were observed in 50% of the cases. In contrast, the yield of a significant decrease in titres among subjects with clinical pertussis was 79% (38/48).

Discussion

In this report, we describe an epidemic of pertussis in a religious community of 75 unvaccinated elderly residents, and 19 vaccinated and five unvaccinated personnel.
The attack rates of clinical pertussis and laboratory-confirmed pertussis were as high as 49% and 42%, respectively. Unvaccinated subjects were more at risk of pertussis than those vaccinated. The rates, the duration, and the severity of laboratory confirmed pertussis tended to increase with age. The observed pertussis-associated mortality was 5% (4/75) in the unvaccinated elderly residents.

One hundred percent of the population (n = 99) participated in the study. The epidemic was reported late to the public health department because the clinicians of the institute did not consider pertussis to be a possible cause of the epidemic. As a result, clinical symptoms had to be studied retrospectively for a 9-week period. However, retrospective clinical data collection over a 9-week period in infectious disease epidemics seems to be reliable.[15]

Another limitation of the partly retrospective study is that sample collection occurred relatively late during the epidemic. As a result, in the majority of subjects, the yield of the golden standard tests to confirm the diagnosis of clinical pertussis was low (PCR, culture, significant increase in antibody titre). Previously, it has been described that the percentage of positive culture can be as low as 1–2% three weeks after the onset of cough. At that time, the recovery by means of PCR is below 10%.[10] In addition, among the subjects with clinical pertussis, concordant results between a reactive PCR, a positive culture, and a significant increase in antibody titres occurred infrequently. The low yield of these tests is a generally

accepted problem in the diagnosis of pertussis. In contrast, retrospective analysis by a significant decrease in titres among subjects with clinical pertussis was as high as 79% when using a third serum sample at week 60 of the epidemic.
Prolonged cough and cough at night were the most common clinical symptoms among the unvaccinated elderly with laboratory-confirmed pertussis. Previously, Keitel and Adwards[16] described that, in adults, the most common manifestation of pertussis is prolonged cough.
In the early years of the 20th century, it was observed that only 2.3% of cases during epidemics of pertussis occurred in persons older than 15 years and that children could be infected by adult household contacts.[17,18] In more recent years, the number of cases with pertussis among older children and adults increased, and the proportion of reported cases among persons older than 10 years increased from 15.1% in 1977 to 27% in 1993.[19] Among adults, a high prevalence of pertussis is described in recent reports[6,7] although, compared with this study, the prevalence in elderly was found to be relatively low.[4]

Three of the four pertussis-associated deaths developed symptoms of intracranial bleeding during the coughing period, possibly as a result of increased intracranial pressure. This complication of pertussis has been described previously.[20,21] Intracranial bleeding could possibly be a significant cause of death in elderly persons with pertussis during a prolonged period of coughing. To our knowledge, this is the first time that such a high pertussis-associated mortality rate has been reported among the elderly. In the USA, in 1992 and 1993, only 23 pertussis-related deaths were reported. They all occurred in children, who most frequently died as a result of pneumonia.[22] Thirteen percent of these children demonstrated signs of encephalopathy.

In conclusion, an epidemic of pertussis among unvaccinated adults is described, with attack rates tending to increase with age. Among the elderly, an unexpectedly high attack rate of pertussis was observed. Four of six deaths are thought to be associated with pertussis. Therefore, physicians should be alert to the diagnosis of pertussis in unvaccinated elderly with prolonged and nightly cough.

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chapter

3

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

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Summary

To investigate the possible dependence on age of the rate of decline of IgG antibodies to pertussis toxin (IgG-PT) after natural infection with *Bordetella pertussis*, we measured IgG-PT in follow-up sera of 121 patients (age 0–94 years) obtained after 123 episodes of *Bordetella pertussis* infection.

For analysis we applied a dynamic model for the inactivation of *Bordetella pertussis* by the immune system.

There were no significant differences in rise, peak and decline of IgG-PT between different age groups, although there was a tendency for a more rapid increase, a higher peak and a faster decline with increasing age.

The IgG-PT cut-off of 100 U/ml for serodiagnosis of pertussis appeared valid in all age groups.

A decline of IgG-PT to <10 U/ml was associated with increased risk of re-infection with *Bordetella pertussis*.

Introduction

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

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

In our first study of the longitudinal course of IgG-PT after typical symptomatic infection with *Bordetella pertussis*, practically all 57 participating pertussis patients were young children and an established model for assessing the rate of decline of IgG-PT antibodies was lacking.[19] For assessment of the possible age dependence of the rate of IgG-PT decline after natural infection, we extended our studies to a larger group of pertussis patients of various ages for whom follow-up sera were available, and to a group of elderly adult patients with pertussis for whom a follow-up serum sample had been drawn 1 year after infection with *Bordetella pertussis*.[23] For analysis we applied an adapted version of a recently described dynamic model for the inactivation of *Bordetella pertussis* by the immune system.[24]

Materials and Methods

Patients and sera

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

The second group (B) of pertussis patients studied consisted of 34 people from a convent in the southern part of The Netherlands, 33 nuns (aged between 59 and 94 years) and one employee (aged 26 years). All of them had clinically typical pertussis during an outbreak of pertussis in this convent in 1992, which was confirmed by positive IgG-PT serology (for criteria see below). This pertussis outbreak was described in detail previously.[23] Approximately 1 year after the outbreak one follow-up serum sample had been obtained from all patients. All patients gave informed consent.

Laboratory evaluation

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

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

Recently, the IgG-PT ELISA used here[19,26] has been compared to an internationally standardized IgG-PT ELISA which was recommended by the United States' Food and Drug Administration (FDA) for use in clinical trials of pertussis vaccines, and in which the 'lot 3 pertussis serum' of the FDA, with a predefined content of IgG-PT in IU/ml, is used as reference serum.[27–29] It was shown by Giammanco et al.[29] that there was a good correlation between both assays and that the relation between IU/ml in the internationally standardized ELISA and U/ml in our ELISA as calculated through linear regression of log-transformed concentrations with the formula $\log_{10}(U/ml) = 0.2174 + 0.8475\log_{10}(IU/ml)$. This results in 5 U/ml (detection level) = 3.7 IU/ml and 100 U/ml (diagnostic cut-off) = 125 IU/ml. [29]

Data analysis

IgG-PT responses show considerable variation among individual patients, as is clear in Figure 1. Therefore we analyse these responses with a hierarchical

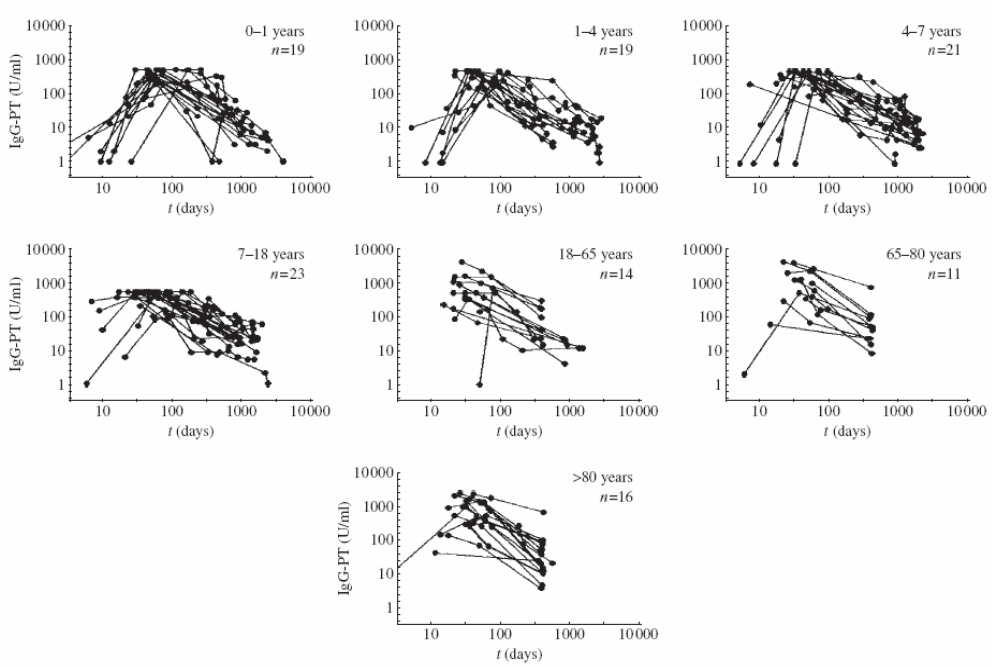

Fig. 1. Measured IgG-PT levels against time from infection [log10 (IgG-PT) against log10 (time)]. Different age groups are in separate panels; data from individual patients are connected.

model, allowing for variations between individual patients. For any individual patient the amount of information is limited: only a few measurements at best. For that reason we have described longitudinal responses with a model of the interaction between bacteria and the immune system. The model assumes that during infection bacteria are growing exponentially in the host. At the same time, bacteria are inactivated (killed) by antibodies (or by some action associated with the antibody response) according to a mass-action mechanism: inactivation depends on the product of the concentrations of bacteria and antibodies (or antibody-producing cells). Conversely, antibody production is controlled by the probability of antibodies (or antibody-producing cells) encountering bacteria (or being presented with antigens derived from these pathogens). Antibody removal is considered an autonomous first-order process. This is the simplest possible model for the interaction between host and pathogen, and it is well known in population biology as a Lotka–Volterra model.[30] The model can be formulated as a system of ordinary differential equations (see Appendix).

This set of equations can be solved numerically and the solution is used for fitting to the patient data. Rather than fitting the model to data from any patient

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

Results

Descriptive analysis.

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

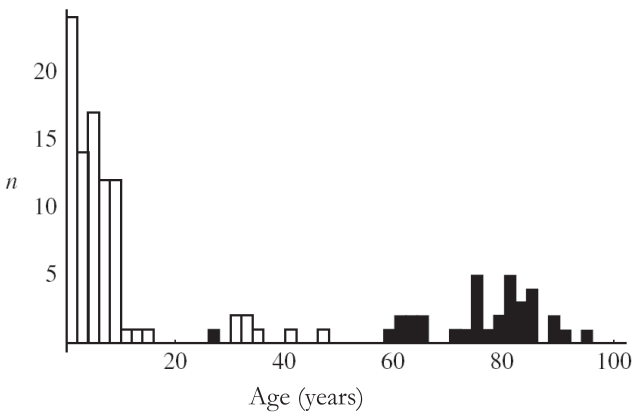

Fig. 2. Age distribution of pertussis cases from the two studies (note that these are 2-year categories, different from the age categories used for analysis). Patients<18 years are from group A (□), those >65 years from group B (■). Adults (18–65 years) are either parents of children in group A, or from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

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

The age distribution of the 34 patients of group B is also given in Figure 2. Since the national vaccination programme in The Netherlands against pertussis started in 1952, the 33 patients of group B who were born before 1952 (i.e. all the nuns) had not been vaccinated. The one patient born after 1952 (the employee) had been completely vaccinated. From the 34 pertussis episodes of the 34 patients of group B 99 sera were available, with 64 sera in the first 6 months after onset, six sera from six patients in the second half year, and 29 sera from 29 patients in the second year (Table 1). The mean number of sera per patient/episode was 2.97 (range of 2–3). The time between onset of pertussis and obtaining the last follow-up serum sample ranged from a minimum of 11.1 months to a maximum of 13.6 months, with a median of 12.6 months.

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

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

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

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

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

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

Re-infections

In the three patients in group A with re-infection after 3.4, 5.8 and 6.6 years, the maximum IgG-PT titre in the first months after the first typically sympto-

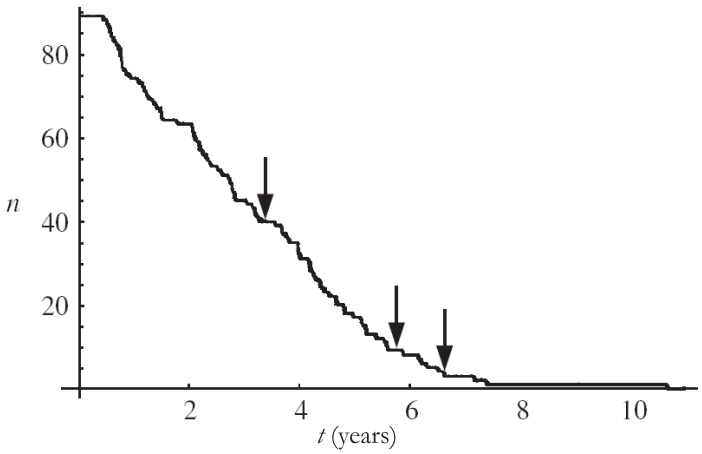

Fig. 3. Number of times patients of group A have been in the study; numbers of patients as a function of the time between onset of disease and last serum sample. The arrows denote time points of confirmed re-infections (one at each time point).

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

In Figure 3 the times (after first infection) of occurrence of the three re-infections have been indicated. It can be seen that the one re-infection after 3.4 years was among 40 patients with a follow-up of 3.4 years or more (2.5%), the one re-infection after 5.8 years was among nine patients with a follow-up of 5.8 years or more (11%) and the one re-infection after 6.6 years was among four patients with a follow-up of 6.6 years or more (25%).

Analysis in the dynamic model

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

Figure 1 shows the individual responses of IgG-PT to infection with *Bordetella pertussis* in the seven different age groups. In age groups (a)–(d) and part of group (e) (i.e. the 7 patients derived from group A) the IgG-PT concentrations are censored at an upper level of 500 U/ml (see "Laboratory evaluation" section above). In part of group (e) (i.e. the 7 patients derived from group B) and in groups (f)–(g) the non-censored IgG-PT concentrations are given. The regression model allows for censoring of IgG-PT data from group A (as reported previously[24]), making groups A and B comparable. Note that in Figure 1, both, IgG-PT concentrations and time elapsed since onset of pertussis, are logtransformed. In this manner both the rapidly increasing as well as the slowly decreasing phase of the immunoresponse can be shown in a single graph.

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

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

	Time to peak			Time to 100 U/ml			Time to 5 U/ml		
Age (years)	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$	$Q_{0.05}$	Q0.50	$Q_{0.95}$
0-1	0.55	1.18	2.94	1.34	8.28	38.90	15.11	36.48	104.36
1-4	0.47	1.45	2.72	1.48	5.30	97.83	4.63	26.27	257.39
4-7	0.41	0.96	1.86	1.80	14.06	34.40	21.74	62.06	159.55
7-18	0.38	0.77	1.63	3.12	12.25	29.09	20.11	47.93	113.90
18-65	0.09	0.31	1.34	2.29	8.73	25.23	4.56	23.16	100.32
65-80	0.14	0.45	1.20	2.73	10.63	24.61	8.63	25.48	75.67
≥ 80	0.15	0.40	1.52	2.08	6.61	19.44	3.75	17.13	68.55

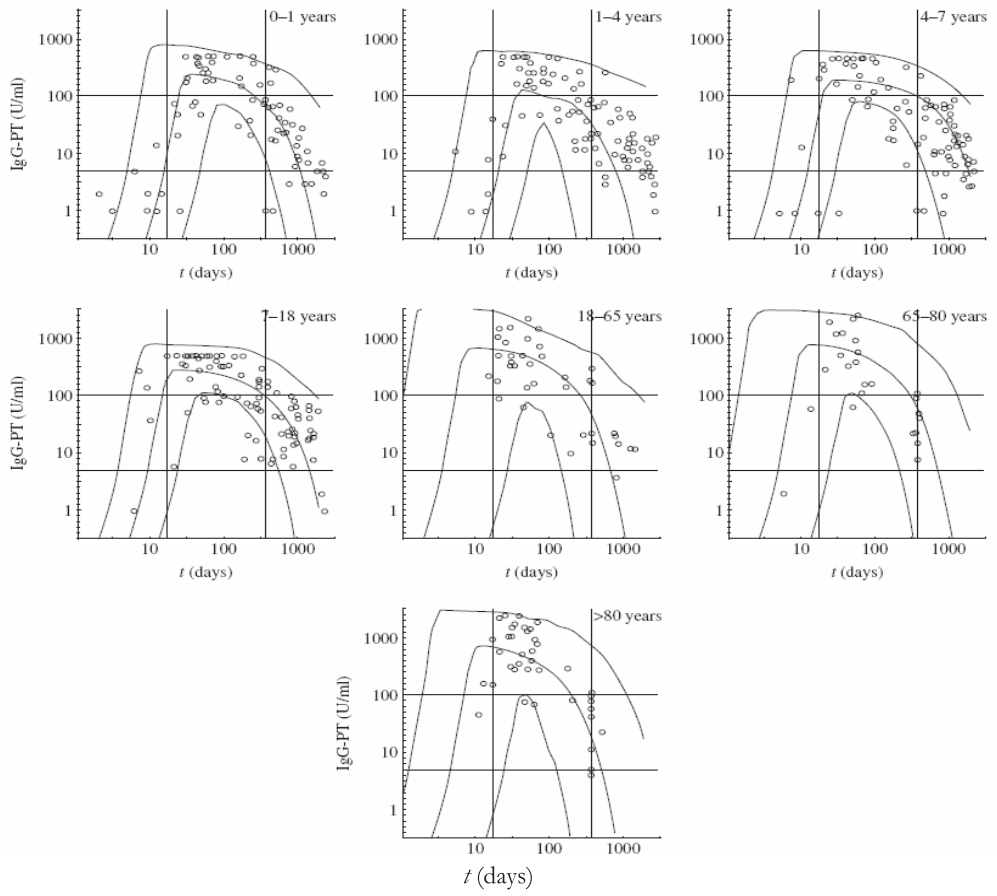

Fig. 4. Predicted IgG-PT antibody responses to infection in each of the age groups, on a $\log_{10}$ (IgG-PT)-$\log_{10}$ (time) scale to show both initial rise and final decline of the responses. Intervals show (95%) range of variation in response among individual patients. Circles indicate observed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

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

Age (years)	Peak titre (U/ml)		
	$Q_{0.05}$	$Q_{0.50}$	$Q_{0.95}$
0-1	85.9	244.3	624.7
1-4	46.2	136.2	575.1
4-7	100.3	213.0	621.6
7-18	124.3	303.0	653.7
18-65	138.7	695.2	2631.6
65-80	188.2	792.6	2384.6
≥ 80	162.1	720.5	2503.3

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

The estimated variation in the older groups (e)–(g) is higher than in groups (a)–(d). While it cannot be excluded that this is also – in part – caused by the influence of censoring, it should also be noted that the records of older patients (of group B) consist of fewer measurements (two or three) than those of the juvenile patients (of group A). We checked the influence of censoring by recalculation after arti-

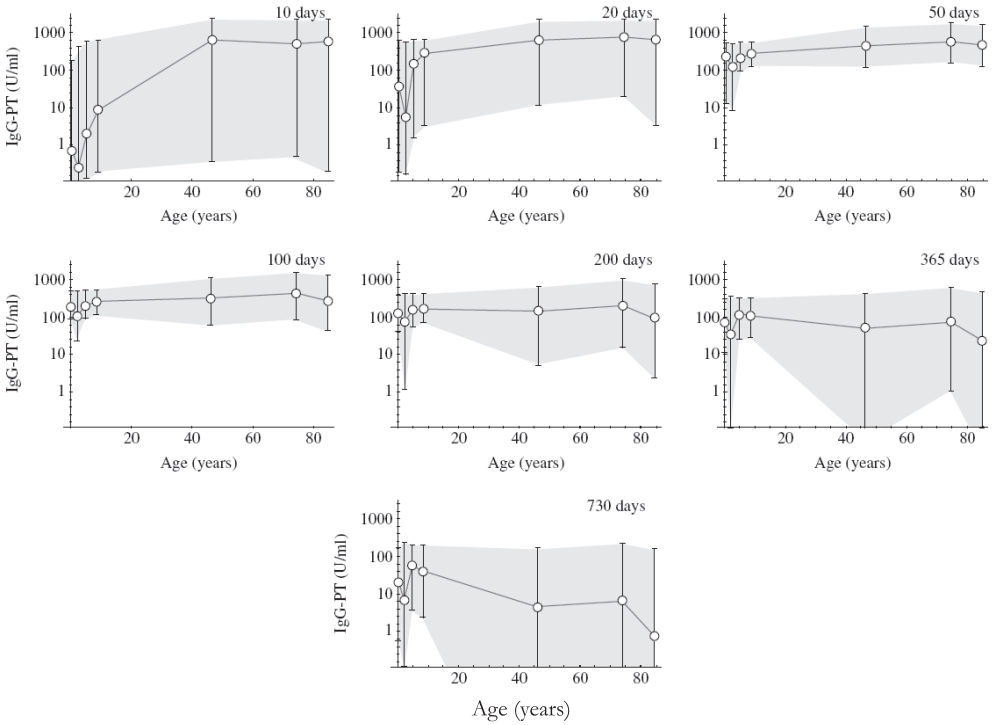

Fig. 5. Age profiles of (log_{10}) IgG-PT responses : predicted antibody levels against age (category) at various times from infection. As in Figure 4 the intervals show (95%) range of variation in response among individual patients.

ficially censoring the data from group B, finding similar results, confirming that correction for censoring of the data from group A in the dynamic model was appropriate (data not shown).
In Figure 5 the predicted age profile of IgG-PT responses at different time-points after onset of pertussis is given (10, 20, 50, 100, 200, 365 and 730 days). At 10 and 20 days there is large variation in response in all age groups, possibly partly due to large variations in the rapidity of the increasing phase of the IgG-PT response to infection, and possibly partly due to inaccurate reports of the first day of the disease. However, at both time-points the IgG-PT concentrations in the three adult subgroups tended to be higher, suggesting that the IgG-PT response to infection in adults may be somewhat more rapid. At 50 and 100 days all predicted median IgG-PT levels are >100 U/ml and the variation of responses is small, without substantial differences among age groups, although peak responses in the oldest two subgroups appeared to be somewhat higher. At 200 days antibody decay has started in all age groups.

In the older patients (65 to < 80 and ≥ 80 years) there seemed to be a tendency towards a more rapid decline of IgG-PT after 365 and 730 days than in younger patients. The second youngest age group (1–4 years) appears to show a response, which is slightly different from the other age groups (slower increase, lower magnitude). As already stated, data in this group show markedly high dispersion, as may also be appreciated by comparing error bars (illustrating variation) in Figure 5 (see also Fig. 4 and Tables 2 and 3).

Discussion

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

Our study is unique with respect to the high number of participating patients, the wide age range of the patients, the long follow-up times in part of the patients, and also the method of analysis, using a hierarchical model for induction and decay of antibodies after infection. The few studies of the course of IgG-PT after infection with *Bordetella pertussis* that have been published all show decline over time, but assessment of possible differences in children and adults is lacking and follow-up times are relatively short. In the studies of Hodder et al.[9] and Heininger et al.[32] the course of IgG-PT over a period of 20 months after pertussis in 48 adults aged > 65 years[9] and over a period of 28 months in 11 adults[32] was remarkably similar to the pattern over the same time span in adults in our study. In those studies the internationally standardized IgG-PT ELISA, recommended by the FDA was used, of which the relationship with our ELISA is known (see Materials and Methods section.[29]). For instance, the geometric mean IgG-PT titre of 11 adult pertussis patients was 242 IU/ml at 2 months after onset of infection and 45 IU/ml at 28 months after onset.[32] However, in none of those patients did IgG-PT decline below detection levels, which may be related to the limited follow-up time. Two other studies, one comprising both adults and children[2] and one comprising young children,[33] also showed a significant decline of geometric mean IgG-PT titre 1 year after pertussis. The study with the longest follow- up time was from Tomoda et al. who measured IgG-PT during an outbreak of pertussis in a semi-closed adult community and again 5 years later.[34] He showed that in the large majority of 21 pertussis patients in that outbreak (37.5 $\pm$ 12.1 years) IgG-PT was > 50 U/ml several weeks after onset of pertussis; 5 years later the IgG-PT in all patients had declined to < 10 U/ml and in most to undetectable levels. These findings support our conclusion that in the majority of cases or perhaps in all cases of pertussis, the subsequent decay of infection-induced IgG-PT does not level off well above detection thresholds but progresses to undetectability.

The relatively short persistence in all age categories of infection-induced peak levels of IgG-PT, supports our previous finding that a diagnostic cut-off of 100 U/ml (equivalent to 125 IU/ml) for serodiagnosis of actual or very recent infection with *Bordetella pertussis* is valid for all ages.[19] Although vaccination can induce IgG-PT levels > 100 U/ml, interference with serodiagnosis of pertussis is minimal because IgG-PT induced by vaccination with wholecell pertussis vaccines or acellular pertussis vaccines also declines quite rapidly. Multiple studies show a decrease after primary and booster vaccination to very low or undetect-

able levels within 1–8 years.[13,35–38] For instance, Taranger et al.[39] followed 813 children after pertussis vaccination with a monovalent pertussis toxoid vaccine. There was a strong IgG-PT response to a geometric mean of 143 IU/ml at 1–2 months after vaccination and a rapid decrease to a geometric mean of 8 IU/ml at 21–32 months after vaccination.

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

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

The three re-infections documented in this study were in children whose IgG-PT at the time of the second infection had declined to < 10 U/ml. Although the number of re-infections was small, their timing and incidence was compatible with the statement that natural infection initially induces protection against

re-infection but that susceptibility to (re)-infection re-emerges when IgG-PT concentrations have fallen to < 10 U/ml and that subsequently, susceptibility for disease in association with infection increases over time. In a previous paper, we have described two other patients who had infection with *Bordetella pertussis* 12 years after the first episode. In contrast to the three re-infections 3.4, 5.8 and 6.6 years after the first in this study, the re-infections after 12 years were associated with typical symptoms, i.e. long-lasting paroxysmal cough.[12]

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

Appendix

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

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

Where:
x(t) = pathogen concentration
y(t) = antibodies
a = pathogen growth rate
b = antibody-dependent pathogen inactivation
c = antibody decay rate
d = pathogen-dependent antibody production rate

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

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

For statistical analysis, parameters were transformed:

$u = \sqrt{ac}$ $\quad w = \sqrt{bd}$
$v = \sqrt{a/c}$ $\quad z = \sqrt{b/d}$

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

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

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Sensitivity and specificity of single IgA and IgG antibody concentrations for early diagnosis of pertussis in adults: an evaluation for outbreak management in public health practice

chapter

4

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Abstract

Background. An accurate, practical laboratory test is needed to confirm clinical diagnosis of pertussis in adults during the first 3 symptomatic weeks, when treatment is effective and transmission can be interrupted.

Methods. The sensitivity and specificity of single IgA and IgG levels were assessed in a cohort study of a pertussis epidemic in 99 adults in a closed community. Sensitivities were assessed in the sera of 46 laboratory confirmed clinical pertussis cases during the first 3 weeks. Specificities were calculated in sera of 35 asymptomatic controls without clinical symptoms or laboratory confirmed infections from the same community (internal controls). We compared these specificities with the specificities of single IgA and IgG levels in 4275 external controls from a cross-section of the general Dutch population aged 21-79 years who had not coughed for more than 2 weeks in the past year, and without pertussis diagnoses. The study was done in the Netherlands when whole-cell pertussis vaccine was used in the national vaccination programme.

Results. Levels of 24 U/ml for IgA and 27 U/ml for IgG gave sensitivities of 100% and 75%, respectively, in the first 2 weeks, 100% in the third week, and 97% after the fourth week. The levels were reached within 2 days after onset of increase, and remained above these levels for roughly 7.2 and 5.1 months, respectively. Specificity was 82% for IgA and 89% for IgG in the internal controls and 90% in the external controls, respectively.

Conclusions. We suggest levels of 24 U/ml for IgA and 27 U/ml (= 27 International Units (IU)/ml) for IgG as sensitive, specific, and practical for laboratory confirmation of clinical pertussis in adults in the first 3 weeks of outbreak management.

Background

Pertussis is a bacterial infection caused by *Bordetella pertussis*. Despite the introduction of mass vaccinations in the Netherlands in 1952 and other countries, pertussis is still an endemic disease with regular epidemic outbreaks.[1-5] In unvaccinated populations, pertussis morbidity and mortality occur predominantly in those 15 years or younger.[6] In vaccinated populations, pertussis is more likely to be found in older children and adults.[7-13] Circulation of *Bordetella pertussis* in vaccinated children, adolescents, and adults plays an important role in the continuing transmission of the pathogen to infants too young to be vaccinated, in whom the disease is the most severe and sometimes fatal.[14-19]

Pertussis is most infectious in its prodromic and early clinical stages. Effective management in unvaccinated infants and during outbreaks requires early diagnosis and treatment of cases, accompanied by antibiotic prophylaxis of contacts.[20,21] There is no evidence of benefit from chemoprophylaxis given more than 21 days after the onset of the primary case.[22,23] Therefore, antibiotic management of pertussis should be initiated promptly to minimize secondary spread.[24] For the improvement of clinical diagnosis and pertussis surveillance, rapid, easily accessible, highly sensitive and specific laboratory methods are needed to diagnose pertussis in the first 3 weeks.[25]

The validity of methods for pertussis diagnosis depends on the time of initiation. Early in the disease, culture and Polymerase Chain Reaction (PCR) can be used. Culture of *Bordetella pertussis* is highly specific, but laborious and insensitive.[26] The yield progressively decreases during disease[27] and is low after 2 weeks and antibiotic use.[28] The sensitivity of PCR is superior, but rapidly decreases with increasing duration of the disease and patient age.[29,30] Serology is a good alternative.[29,31,32] In this study, we assessed the sensitivity and specificity of single IgA and IgG antibody levels for laboratory confirmation of clinical pertussis in adults during the first 3 weeks of symptoms. The study was done in The Netherlands when whole-cell pertussis vaccine was used in the national vaccination programme.

Methods

Study population

The clinical and laboratory outcomes of a pertussis epidemic in a religious convent, which has been described previously, was used as a sample population to evaluate single IgA and IgG levels after other causes for an outbreak of coughing were excluded.[12]

In short, the epidemic was reported to the Municipal Public Health Service (MPHS) in its 9th week for outbreak management, at which time the study began. The convent was a nursing home for 75 elderly resident nuns supported by 24 non-resident personnel. The personnel consisted of 3 males and 21 females. The mean age was 75 years (range 55-94 years) for the nuns and 27 years (range 21-46 years) for the personnel. All of the nuns and 5 of 24 non-resident personnel had never been vaccinated against pertussis. The nuns and staff shared common social and religious activities, and shared meals in the dining room. Pertussis cases were cared for in the convent by nuns and personnel. Retrospective interviews based on structured questionnaires were conducted to obtain information about the onset of characteristic clinical symptoms in first 9 weeks of the epidemic.

The emergence and duration of the clinical manifestations were prospectively monitored daily from week 9 up to week 13, the period that the epidemic persisted with new cases. After week 13, cases were monitored weekly until symptoms subsided.

Two nasopharyngeal swabs were obtained from each individual at weeks 9 and 13 of the epidemic, one for culture and one for PCR.

At weeks 9, 13, and 60 serum samples were obtained to determine IgA and IgG antibody levels.

Definitions

Laboratory confirmed Bordetella pertussis infection was defined as one of the laboratory findings: 1) *Bordetella pertussis* strain isolated from the nasopharynx; 2) reactive PCR; 3) significant (at least 4-fold) increases or decreases[33,34] of IgA or IgG antibody levels between at least one pair of serum samples obtained at weeks 9, 13, and 60 of the epidemic; and 4) IgG level ≥ 100 U/ml at weeks 9 or 13 of the epidemic[35] (equivalent to 125 International Units (IU)/ml).[12]

We defined *clinical pertussis* as a persistent cough and its duration was divided in "7-13 days" and "at least 14 days".[36]

Pertussis cases had both laboratory confirmed *Bordetella pertussis* infection and clinical pertussis.

The index case was the first pertussis case in the epidemic.
Pre-epidemic cough was defined as a cough that occurred before the emergence of the index case.
Internal controls had no laboratory confirmed *Bordetella pertussis* infection and were asymptomatic residents or non-resident personnel, i.e. they had no pre-epidemic cough nor clinical pertussis.
Sensitivities of different IgA and IgG levels were calculated as the proportion of serum samples with a positive test result. The calculations were done in samples obtained from pertussis cases who had coughed for at least one day during their period of clinical pertussis.
Specificities of different IgA and IgG levels were calculated as the proportion of serum samples with a negative test result. These calculations were done in samples obtained from the internal controls.
Specificities were also calculated in 4275 external controls from a cross-section of the general Dutch population in the same age group (21-79 years) as the convent population. Control subjects reported in a structured questionnaire to have not coughed for more than 2 weeks in the past year, nor to have had a physician-diagnosed pertussis.[35,37] From the external controls, specificities of different IgA and IgG levels were calculated as the proportion of the 4275 serum samples with a negative test result.

The duration of waxing and waning of IgA and IgG concentrations

To estimate the time period after which single IgA and IgG concentrations can be reused as a diagnostic test for a subsequent pertussis infection, we assessed the duration (in days) of waxing and waning of IgA and IgG concentrations after a *Bordetella pertussis* infection. Therefore we first calculated the geometrical mean concentration (GMC, U/ml) over the highest IgA and IgG levels obtained in week 9 and 13 of the epidemic for all subjects with a significant increase or decrease of antibody level. In these subjects we then calculated the mean rate of increase (expressed as U/ml/day) towards the GMC and the mean rate of decrease from the GMC downwards. This was done for IgA and IgG separately.

Laboratory methods

In the two populations, the serological laboratory investigation of pertussis specific IgA and IgG antibodies was performed by enzyme-linked immunosorbent assay (ELISA) at the National Institute for Public Health and the Environment (RIVM), the Netherlands, as described previously.[12,31,35,38] For IgA class antibody detection, a crude cell-wall preparation of *Bordetella pertussis* was used. For IgG

class antibody detection, purified pertussis toxin was used. Antibody binding activities were quantitatively expressed in 'local' units per millilitre (U/ml). IgG can be converted to Food and Drug Administration (FDA) international units (IU) non-linearly by the formula $\log_{10}(U/ml) = 0.2174 + 0.8475\log_{10}(IU/ml)$.[39,40] The detection limit of the assays was 5 U/ml.[35]
In our evaluation of sensitivities and specificities of single IgA and IgG levels, we focused on levels at least 4 times the detection limit.
Culture and PCR were processed as described previously.[12,30,41]

Results

Convent population

The convent population consisted of 75 nuns and 24 personnel. All 99 individuals participated in the study. The pertussis epidemic started in week 1 with the emergence of the first case with laboratory confirmed pertussis infection. The last case was detected in week 13. During the study, 6 residents died resulting in 99, 99, and 93 study subjects in weeks 9, 13, and 60 of the epidemic, respectively.

Clinical outcome

Retrospective evaluation of weeks 1-9 of the epidemic revealed 2 subjects with a pre-epidemic cough, 3 subjects with clinical pertussis of 7-13 days duration, and 41 with clinical pertussis coughing 14 days or more. Prospective evaluation in weeks 9-13 of the epidemic revealed another 6 subjects with clinical pertussis coughing 14 days or more and 47 with no cough (Table 1). Clinical pertussis persisted for a mean period of 69 days (range: 7-268 days).

Laboratory outcome

At weeks 9, 13, and 60 of the epidemic 94, 97, and 85 serum samples, respectively, were obtained. Three samples were collected from 80 subjects, and 2 from 17 subjects. One serum sample was obtained from one resident with, and one resident without clinical pertussis. Of the 99 subjects, 51 showed a significant increase or decrease in IgA or IgG between week 9, 13 and 60 of the epidemic, 5 of whom also had either a positive PCR or culture. Additionally, 7 subjects had a single IgG level of $\geq$ 100 U/ml at week 9 or 13 of the epidemic, with no further significant increase or decrease of IgA or IgG. This resulted in 58 subjects with, and 41 without laboratory confirmed pertussis infection (Table 1).

Pertussis cases for sensitivity and internal controls for specificity calculations

Table 1 shows the source data for the sensitivity calculations, the 46 pertussis cases (45 cases with clinical pertussis coughing 14 days or more and 1 case coughing 11 days, all with laboratory confirmed pertussis infection) and the 35 internal controls who had no cough and no laboratory confirmed pertussis infection. The mean age of the cases and internal controls was 77 years (range: 21-86 years) and 56 years (range: 22-91 years), respectively.

The levels of IgA and IgG obtained from the 46 pertussis cases and the 35 internal controls are shown in Table 2. The GMC of IgA and IgG in the 46 pertussis cases was significantly higher ($p < 0.001$) in all sampling weeks compared to the 35 internal and 4275 external controls.

Table 1. Outcome of clinical and laboratory investigations in absolute numbers in the convent population (N=99).

Outcome of laboratory tests for pertussis infection	Clinical Outcome				Total
	Clinical pertussis coughing > 14 days	Clinical pertussis coughing 7-13 days	Pre-epidemic cough	No cough	
	(n=47)[c]	(n=3)[c]	(n=2)	(n=47)[e]	(N=99)
Laboratory confirmed pertussis infection	45[b]	1[b]	0	12[f]	58
IgA or IgG 4-fold increase or decrease between week 9, 13 and 60 of the epidemic	41	1	0	9	51
IgG ≥ 100 U/ml at week 9 or 13 of the epidemic, no IgA or IgG 4-fold increase or decrease	4	0	0	3	7
Details of laboratory confirmed pertussis findings					
- IgG ≥ 100 U/ml	*41*	*0*	*0*	*6*	*47*
- IgA 4-fold increase	*8*	*1*	*0*	*2*	*11*
- IgA 4-fold increase or decrease	*22*	*1*	*0*	*5*	*28*
- IgG 4-fold increase	*6*	*1*	*0*	*2*	*9*
- IgG 4-fold increase or decrease	*39*	*1*	*0*	*9*	*49*
- PCR or culture positive[a]	*5*	*0*	*0*	*0*	*5*
No laboratory confirmed pertussis infection	2	2	2	35[d]	41

[a] All culture and PCR positive subjects had a significant increase of IgA or IgG as well

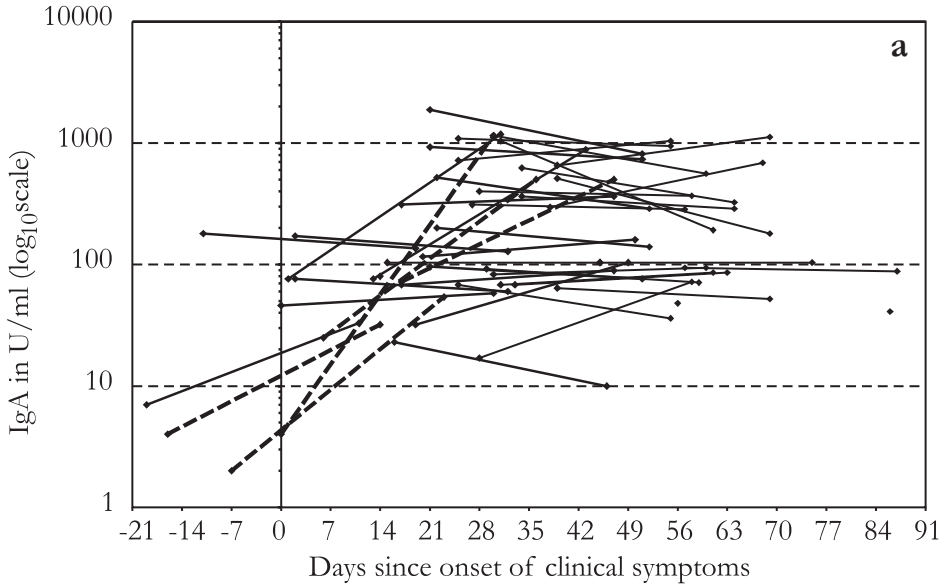
[b] Pertussis cases for sensitivity calculation

[c] Subjects for calculations of spectrum bias in sensitivity

[d] Internal controls for specificity calculation

[e] Subjects with no cough for calculations of spectrum bias in specificity

[f] Subjects with asymptomatic pertussis infection



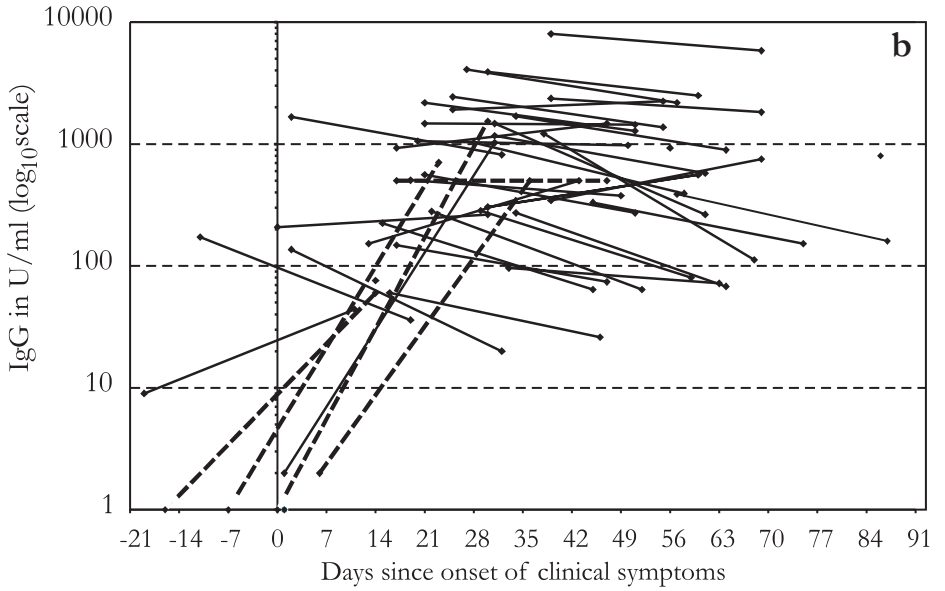


Figure 1a and 1b. The course of IgA and IgG. The course of IgA (Figure 1a) and IgG (Figure 1b) levels obtained in all 89 serum samples from the 46 pertussis cases obtained in week 9 and 13 of the pertussis epidemic in the convent population (n=99). Pertussis cases had a laboratory confirmed *Bordetella pertussis* infection and a clinical pertussis (see methods).
The levels are related to the first day of cough of the pertussis cases. Six serum samples were obtained from cases before they started coughing and 83 samples were obtained from cases who had been coughing for between 1 and 87 days. Lines connect samples obtained from one subject. Dotted lines are from the 5 subjects with a positive culture or PCR.

Course in time of IgA and IgG in pertussis cases

Figure 1 shows the longitudinal courses of the 89 IgA and IgG concentrations obtained from the 46 pertussis cases in weeks 9 and 13 of the epidemic, as related to the onset (day 0) of the clinical period (coughing). Six pre-clinical and 83 clinical serum samples were obtained from the 46 pertussis cases. The earliest serum sample was obtained 19 days before, and the latest one 87 days after the onset of cough.

The general pattern of IgA (Figure 1a) shows a more consistent increasing and decreasing pattern compared to IgG (Figure 1b). IgG concentrations reached higher levels than IgA concentrations and tended to decrease more rapidly to relatively lower levels earlier in the clinical period compared with IgA. From 3 of the 5 PCR or culture positive cases (dotted lines in the figures), the first serum samples were obtained before the onset of the cough. These 3 cases showed at least 4-fold increases of IgA and IgG levels, starting from below 5 U/ml.

Sensitivity and specificity of single IgA and IgG levels for estimating optimal cut-off values

Figure 2 shows the sensitivities of single IgA and IgG levels calculated in the 83 serum samples obtained in week 9 and 13 of the epidemic from the 46 pertussis cases who had been coughing for at least one day during their period of clinical pertussis.

Sensitivities of different levels of IgA (24-74 U/ml) and IgG (27 - 90 U/ml) ranged from 98% to 75% and 95% to 81%, respectively. The sensitivity of IgG was 80%

Table 2. The Geometrical Mean Concentration (GMC), with 95% CI, of serum samples from pertussis cases and from internal controls at week 9, 13 and 60 of the epidemic, and of the serum samples from the external controls (n=4275).

Subjects and time of sampling during the pertussis epidemic	Number of samples	IgA		IgG	
		GMC	95% CI	GMC	95% CI
Cases (n=46)					
- week 9	43	123	75 – 202	252	119 - 535
- week 13	46	172	121 – 244	355	235 - 537
- week 60	40	60	40 – 89	34	22 – 53
Internal controls (n=35)					
- week 9	34	9.4	6.4 - 13.7	6.0	3.9 – 9.3
- week 13	34	7.8	5.2 – 11.6	3.8	2.4 – 6.1
- week 60	29	6.6	4.4 – 9.9	2.8	1.8 – 4.2
- week 9, 13 and 60 combined	97	7.9	6.3 – 9.9	4.1	3.1 – 5.3
External controls (n=4275)	4275	8.5	7.9 – 9.1	7.4	6.7 – 8.3

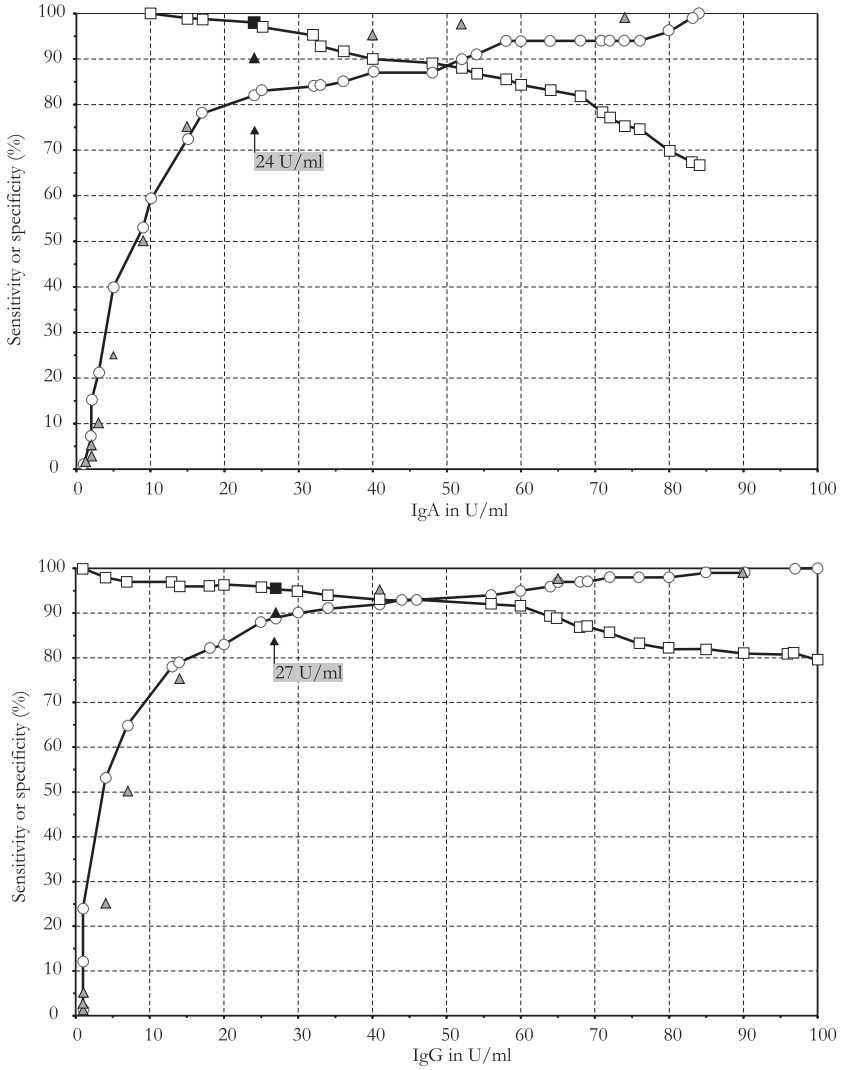


Figure 2. Sensitivity and specificity of different IgA (Figure 2a) and IgG (Figure 2b) antibody levels (U/ml). To estimate optimal cut-off levels, antibody levels against Bordetella pertussis from the convent population during the pertussis epidemic are used.

Sensitivities (-□-) are calculated in the 83 serum samples obtained in week 9 and 13 of the epidemic, from the pertussis cases (n=46) who had been coughing for between 1 and 87 days of their total period of clinical pertussis. Pertussis cases had a laboratory confirmed Bordetella pertussis infection and a clinical pertussis (see methods).

Specificities (-o-) are calculated in the 68 serum samples obtained in week 9 and 13 of the epidemic from the internal controls (n=35).

Specificities (▲) of IgA and IgG (levels calculated in the sera (n = 4275) of external controls (n = 4275) are indicated as well.

The sensitivity mark (-■-) of the IgA level of 24 U/ml, 24 and the IgG level of 27 U/ml, 27 in pertussis cases and the corresponding marks with 90% specificity in the external controls (▲) are indicated as well.

at 100 U/ml and 71% at 200 U/ml (not shown in Figure 2). Compared to IgG, sensitivities of IgA started higher but decreased faster with increasing levels.

To probe spectrum bias[42] in the sensitivities obtained in our 46 pertussis cases, we also calculated sensitivities in the 91 IgA and 91 IgG levels obtained in weeks 9 and 13 of the epidemic of all 50 subjects with clinical pertussis, after they had coughed for at least 1 day during their period of clinical pertussis (Table 1). This resulted in lower sensitivities of IgA levels (24-74 U/ml) and IgG levels (27-90 U/ml) ranging from 90% to 69% and from 87% to 73%, respectively, while the sensitivity of IgG of 100 U/ml decreased from 80% to 72%. The lower sensitivities can be explained by the addition of concentrations from three subjects with clinical pertussis who had been coughing for 7, 11, and 21 days, respectively, and with IgA and IgG levels of at most 14 U/ml and 4 U/ml, respectively, suggesting that these three subjects did not have pertussis infection. The addition of a fourth subject increased the sensitivities. Indeed, this was most probably a missed pertussis case with 255 days clinical pertussis and highest obtained IgA and IgG levels of 116 and 80 U/ml, and a 3.6-fold decrease of IgG, while we

Table 3. Specificities in external controls (n = 4275) and sensitivities in time intervals (in days) in reference to the onset of cough in pertussis cases (n = 46) of single IgA and IgG concentrations (see Figure 2). Geometrical Mean Concentrations (GMC) and number of samples are indicated.

Level (U/ml)[a]	Specificity external controls	Sensitivity (%) in time intervals with reference to the onset of cough				
		-21 to 0 d	1 to 14 d	15 to 21 d	22 to 87 d	1 to 87 d
IgA						
24	90.0%	33	100	100	97	98
40	95.0%	33	63	82	95	90
52	97.5%	17	63	82	92	88
74	99.0%	17	63	64	78	75
GMC (U/ml)		11	60	136	212	177
IgG						
27	90.0%	33	75	100	97	95
41	95.0%	33	75	91	95	93
65	97.5%	33	50	82	94	87
90	99.0%	33	38	82	86	81
100[b]	99-100%	33	38	82	84	80
GMC (U/ml)		8	48	390	506	391
No. samples		6	8	11	64	83

[a] Levels are obtained from the estimation of optimal cut-off values in Figure 2

[b] Used by the National Institute for Public Health and the Environment in The Netherlands for surveillance

used a 4-fold change in our case definition. A 3-fold change has been accepted as significant in a sufficiently precise assay.[43]

The specificities calculated in the 68 concentrations in serum samples obtained from the 35 internal controls in the convent population at weeks 9 and 13 of the epidemic are shown in Figure 2. Specificities of different levels of IgA (24-74 U/ml) and IgG (27-90 U/ml) levels ranged from 82% to 94% and 89% to 99%, respectively. The specificity of IgG was 100% at 100 U/ml.
The specificities of different levels of IgA (24-74 U/ml) and IgG (27-90 U/ml) from 4275 sera samples from the 4275 external controls ranged from 90 to 99% (Figure 2). In the external controls IgA levels showed higher specificities than similar IgG levels.

Table 4. Estimated waxing and waning of IgA and IgG levels between diagnostic parameters.

Variable	IgA	IgG
Laboratory detection limit	5 U/ml	5 U/ml
100% sensitivity level in the 46 pertussis cases	24 U/ml	27 U/ml
90% specificity level in 4275 external controls	24 U/ml	27 U/ml
99% specificity level in 4275 external controls	74 U/ml	90 U/ml
GMC of the highest level obtained in week 9 and 13 of pertussis cases with at least 4-fold increase or decrease between week 9, 13 and 60 of the epidemic	229 U/ml (n = 28 levels)	406 U/ml (n = 49 levels)
Average speed of significant increase	16.0 U/ml/day (n = 11 level pairs)	14.7 U/ml/day (n = 9 level pairs)
Average speed of significant decrease	1.0 U/ml/day (n = 18 level pairs)	2.9 U/ml/day (n = 46 level pairs)
Mean time to increase from detection limit to 100% sensitivity level	1.2 days	1.5 days
Mean time to increase from detection limit to 99% specificity level	4.3 days	5.8 days
Mean time to increase from detection limit to GMC	14.0 days	27.3 days
Mean time to increase from 99% specificity level to GMC	9.8 days	21.5 days
Mean time to decrease from GMC to 99% specificity level	156.0 days	109.0 days
Mean time spent above 99% specificity level	165.8 days (5.5 month)	130.5 days (4.3 month)
Mean time to decrease from 99% specificity level to 100% sensitivity level	50.0 days	21.7 days
Total time spent going up and down between detection limit and GMC	238.0 days	165.6 days
Total time spent going up and down between 100% sensitivity level and GMC	217.8 days (7.2 months)	156.5 days (5.1 months)

To probe spectrum bias in the specificities obtained in our 35 internal controls, we calculated specificities as well in the 90 IgA and IgG concentrations obtained in weeks 9 and 13 of the epidemic of all 47 subjects with no cough (Table 1). This resulted in lower specificities of different levels of IgA (24-74 U/ml) and IgG (27-90 U/ml) ranging from 71% to 85% and 72% to 90%, respectively. The specificity of 100 U/ml IgG decreased from 100% to 92%. The lower observed IgA specificities were due to the high levels from the 12 added subjects with a laboratory confirmed pertussis infection and without a pre-epidemic cough or a clinical pertussis. Indeed, 15 of the 22 IgA levels from these 12 subjects were at least 24 U/ml, and of the IgG levels, 18 of 22 were at least 27 U/ml, and 8 were at least 100 U/ml. So the added 12 subjects were not a true control group of unexposed and uninfected subjects.

Sensitivities at different time intervals

Table 3 shows the sensitivities of IgA (24-74 U/ml) and IgG (27-90 U/ml) levels calculated from the 46 pertussis cases (see Figure 2) at different time intervals in reference to the first day of coughing. In the first two weeks after the onset of clinical pertussis, cut-off levels of 24 U/ml for IgA and 27 U/ml for IgG showed sensitivities of 100% and 75%, respectively. In the third week of infection, a sensitivity of 100% was calculated for both parameters. From the fourth week onwards, the sensitivity remained at 97%.

Waxing and waning of IgA and IgG between diagnostic parameters

It was estimated that IgA reached the 100% sensitivity and 99% specificity levels earlier than IgG. IgG waned more quickly back towards pre-infection levels. IgA and IgG remained above their 100% sensitivity level during 7.2 and 5.1 months, respectively and above their 99%-specificity level during 5.5 and 4.3 months, respectively (Table 4). The rate of increase of IgA and IgG levels indicates that the paired samples should be collected within 4 and 7 days, respectively, after they begin to increase in order to observe the fourfold rise towards their GMC.

Discussion

Early diagnosis of pertussis in adults for outbreak management requires low cut-off levels for single IgA and IgG serological tests. We found that cut-offs of 24 U/ml for IgA and 27 U/ml for IgG led to a specificity of 90% and a sensitivity of 100% and 75%, respectively, during the first 2 weeks of pertussis. In the third

week, the sensitivity was 100% for both tests. The sensitivity decreased slightly to 98% for IgA, and 95% for IgG during the first 87 days of clinical pertussis. After acute onset of pertussis, IgA remained above 24 U/ml for a mean duration of 7.2 months, and IgG remained above 27 U/ml for a mean duration of 5.1 months.
Although this study is limited by the relatively small number of subjects, the results are based on a pertussis epidemic in a defined community, with 100% participation. We are not aware of another study in which sensitivities of single IgA and IgG were evaluated in both the pre-clinical and clinical phases of pertussis.

The definition of pertussis was partly based on single IgA and IgG levels, which were also evaluated as diagnostic marker. This may have caused some incorporation bias.[42,44] However, 42 of the 46 pertussis cases were based on at least 4-fold changing IgA or IgG levels in paired samples (Table 1). The lowest IgG level of the 4 cases identified with a single IgG sample was 376 U/ml and they had coughed between 44-263 days. From 3 of these 4 cases we were not able to obtain a third serum sample in week 60 (2 subjects died earlier with pertussis), in order to detect significant change. The fourth subject showed a 3.6-fold changing IgG level. In addition, we did not find other causes of this epidemic of cough[12] in the ideal epidemic circumstances of a convent population with positive cultures for *Bordetella pertussis*.

In our evaluation of spectrum bias in sensitivity and specificity, we showed that our choice of pertussis cases and controls was sound. Arguably, the use of our 35 internal controls may have led to underestimation of the specificity of single low IgA and IgG levels. Indeed 7 of the 35 internal controls had high IgA levels ranging from 24–74 U/ml and 2 internal controls had IgA levels above 74 U/ml. Six internal controls had IgG levels ranging from 27–90 U/ml and one internal control above 90 U/ml. We argue that IgA levels above 24 U/ml and IgG levels above 27 U/ml decreased the specificities obtained in the internal controls, compared with the specificities obtained in the external controls (Figure 2). Among all 47 subjects with no cough (Table 1), 21 (45%) had high single IgA and/or IgG levels. Because these 21 subjects with high single IgA and/or IgG levels had a serological indication of infection (antibody boosting) without symptoms, we consider the external control group, with GMC of IgA and IgG levels significantly lower than in the 46 pertussis cases, the better choice for calculating specificities.

The retrospective part of the study may have resulted in recall bias regarding onset of cough. The study however, was supported by a nun who was responsible for looking after all the nuns with pertussis during the epidemic. This registered nurse kept reliable clinical records including the history of coughing before and after the start of the study. We are therefore fairly confident that recall bias is limited.

IgA antibodies to *Bordetella pertussis* antigens in whole-cell sonicate is known to lack specificity compared to IgA antibodies to pertussis toxin.[30] Indeed single high values of IgA and IgG antibodies to pertussis toxin indicate infections in adults, and IgA is more indicative of a recent antibody response, although less consistent than IgG.[30] However, we excluded causes of the epidemic other than pertussis and used culture, PCR, and IgG against pertussis toxin to positively identify pertussis cases.

In our external control group, the levels of IgA against whole cell sonicate showed higher specificities than similar IgG levels. In the original Dutch study of IgA antibodies, it was stated that IgA antibodies, which are not induced by vaccination, can be used as a reliable indicator of natural infection with *Bordetella pertussis* in adults within one week of infection,[38] especially if interpreted in connection with clinical findings.[46] On the other hand it has been postulated that because of the prolonged antibody response, IgA is not such a useful marker for recent infection.[38] We estimated however, that IgA reaches 100% sensitivity and 99% specificity level sooner than IgG, and that IgA and IgG remain above the 99% specificity level for a mean duration of 5.5 and 4.3 months, respectively, after the onset of pertussis.

IgG antibodies against the virulence factors pertussis toxin, pertactin, and fimbriae increase and decrease after both natural infection and vaccination.[31,47-49] In bacteriologically proven pertussis cases, IgG antibodies declined more rapidly than IgA.[31] This was confirmed in our study.

IgG levels of at least 25 IU/ml were associated with *Bordetella pertussis* infection in a previous study.[31] In our study, a cut-off level of 27 U/ml for IgG resulted in a sensitivity of 100% the third week after the onset of pertussis symptoms, and remained at 97% up to the 13th week with a specificity of 90%.

We determined a sensitivity of 90% for 50 U/ml IgG and of 80% for 100 U/ml, which is comparable to a previous evaluation that determined a sensitivity of 89% for IgG levels above 50 U/ml and 76% for IgG levels of $\geq$ 100 U/ml.[35] Also, our specificity of 99% for an IgG level of 90 U/ml is comparable to that found in the prior study[35] which concluded that, independent of age, a cut-off level of 100 U/ml IgG showed a specificity of 99-100%. In the prior study, most patients reached IgG levels of 100 U/ml within 4 weeks of disease onset which

persisted for 4.5 months. These findings are in line with our estimates that the IgG level increases from the detection limit (5 U/ml) to 100 U/ml in 6.7 days and persists at this level for 4.2 months.
The rate of IgA and IgG increase underlines the importance of obtaining acute phase samples early in the disease in order to detect a significant increase, and consequently the importance of significantly decreasing levels for ultimately diagnosing pertussis if the first sample is not obtained early in the disease.[33,34] Our outcomes are also in line with a study in which a cut-off point of 94 IU/ml for IgG pertussis toxin, with a sensitivity of 80% and a specificity of 93%, has been proposed.[50] This 94 IU/ml is comparable with 76 U/ml in our study and considerable lower then the 125 IU/ml (=100 U/ml) officially used in the Netherlands. In our study 94 IU IgG pertussis toxin had a sensitivity of 83% and a specificity of 98%.

Our findings for low IgA and IgG levels to diagnose pertussis in outbreak management are supported by findings from a pertussis outbreak in a boarding school in Australia, where IgA against whole-cell sonicate, and IgG against pertussis toxin proved useful for early diagnosis and outbreak management. In that study, it was concluded that the IgG level of 125 IU/ml (100 U/ml) was not sensitive enough to identify pertussis cases in their early stages for outbreak management.[51]

Because IgA is not induced by vaccination against pertussis, it may be preferred over IgG in recently vaccinated subjects, as IgG is induced by vaccination with whole-cell vaccines against pertussis used in the Netherlands.[31] Other vaccines may induce even higher IgG-pertussis toxin levels, since the response to pertussis toxin varies between different whole-cell vaccines and acellular vaccines. These IgG-pertussis toxin levels can reach levels higher than 100 U/ml.[48,52-54]

Conclusions

High sensitivity and specificity are required to track and exclude pertussis in vaccine efficacy trials[43] if another serious disease is suspected, and in passive surveillance systems used to estimate vaccine efficacy. In clinical practice and outbreak situations, diagnosis of *Bordetella pertussis* illness must be immediate to allow for prompt therapeutic intervention to reduce disease severity and spread. Therefore, diagnostic criteria should be sensitive, even if specificity is compromised.[55] Pertussis has recognizable characteristic clinical symptoms to a

physician.[56] A clinical case definition can be used in clinical practice and outbreak management,[57,58] and for antibiotic management.[59]

We conclude that after infection with *Bordetella pertussis* IgA and IgG concentrations start to increase from low levels upwards, and that low cut-off levels of 24 U/ml for IgA antibodies and 27 U/ml (equivalent to 27 IU/ml) for IgG antibodies could be considered as practical tools for laboratory confirmation of clinical pertussis in adults during the first 3 weeks of disease outbreak for public health practice, e.g. outbreak management. We suggest a more extensive study of the value of IgA antibodies to pertussis toxin for the early diagnosis of pertussis.

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chapter

5

A pertussis outbreak associated with social isolation among elderly nuns in a convent

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Abstract

The pertussis incidence during an outbreak in a convent in The Netherlands in 1992 was higher among 75 retired (unvaccinated) nuns (60%) than among 24 staff members (8%) and was higher among 9 nuns with only a convent career (100%) than among 66 nuns who had a career outside of the convent (55%). The pertussis incidence increased with duration of social isolation but not with age.

Introduction

A decline in pertussis incidence was seen starting at the beginning of the 20th century; it became more pronounced in the 1940s, when pertussis vaccine became available.[1] Despite vaccination, pertussis resurged; the resurgence occurred first among children and was related to a decrease in vaccination coverage as a result of opposition, in the 1970s, to the use of whole-cell vaccine. In the 1990s, pertussis was increasingly seen in adults; this was related to waning vaccine-induced immunity in countries with high and uninterrupted vaccination coverage among children.[2,3] The proposed explanations for this re-emergence are genetic changes in *Bordetella pertussis* that make vaccines less effective, lessened potency of vaccines, waning of vaccine-induced immunity, greater pertussis awareness, and general availability of better laboratory tests.[4] In addition, a decreased incidence of natural immunity-boosting infections with *Bordetella pertussis,* as a result of the high coverage of national vaccination programs, might explain the re-emergence of pertussis.[5]

The importance of natural *Bordetella pertussis* infections is not well understood. In this report, we describe an outbreak of pertussis in a convent of retired and unvaccinated nuns. Some had only limited exposure to society during their working life, whereas others had careers outside the convent, in The Netherlands and in the tropics. We aimed to study the role of natural infection by determining the relationship between incidence of pertussis and duration of isolation from society.

Methods

To evaluate the association between pertussis incidence and age, isolation from society, and a career in society, the clinical outcomes and laboratory findings of a previously described pertussis epidemic in a convent were used.[6] The convent population consisted of 75 resident nuns and 24 non-resident staff members. The cohort study started in week 9 of the epidemic, when pertussis was diagnosed by culture, PCR, and serum IgG serological tests, and other causes of respiratory tract infections (e.g., tuberculosis and influenza) were excluded. From week 10 onward, outbreak management with antibiotics was focused on pertussis. The onset of clinical symptoms, such as coughing, was established retrospectively through interviews.

Nasopharyngeal swab samples for culture and PCR in charcoal medium were processed as described elsewhere.[7] Titers of IgG antibody to pertussis toxin were determined by ELISA[8] at the National Institute for Public Health and the Environment, Bilthoven, The Netherlands. Antibody binding activities were quantitatively expressed in "local" units per milliliter, which can be converted to US Food and Drug Administration international units.[9] The detection level of the assay was 5 U/mL. Pertussis infection was defined as the presence of 1 of the following laboratory findings: (1) a *Bordetella pertussis* strain isolated from the nasopharynx; (2) a reactive PCR result; (3) a significant (at least 3-fold) increase or decrease in IgG antibody titers between serum samples obtained at weeks 9, 13, and 60 of the epidemic; or (4) a single IgG titer of $\geq$ 100 U/mL.[8] Clinical pertussis was defined as a persistent cough for at least 10 days, starting after the appearance of the first case of laboratory confirmed pertussis infection. Pertussis was defined as pertussis infection and clinical pertussis combined.

The career files of all 75 nuns in the convent archive and of the 24 staff members were examined, by use of a structured questionnaire, to assess date of entrance into the convent, duration of isolation in the convent, duration of a career in Dutch society or as a missionary in the tropics, and duration of isolation after retirement in the convent up to the start of the outbreak in June 1992.

Statistical associations between pertussis incidence and age and career type were analyzed by use of a Yates-corrected χ^2 test. The relationship between pertussis incidence and age and the total duration of isolation before and after retirement in the convent and after retirement only were analyzed by χ^2 test for linear trend (Epi Info, version 6.04d; Centers for Disease Control and Prevention). Age adjustment was performed by logistic regression analysis (SAS, version 8.2; SAS Institute). The statistical association between median duration of cough and age and duration of isolation was calculated by linear regression analysis with cough duration as outcome (S-PLUS, version 6.0; Insightful).

Results

Since entry into the convent (the first of the nuns entered in 1922), all 75 nuns had been based in the same convent of the congregation of the Sisters Servants of the Holy Ghost in The Netherlands. Nine of the 75 nuns stayed in the convent for the rest of their lives after entry. Nuns slept in separate rooms, shared their meals in the dining hall, and only seldom left the convent or received visitors.

The other 66 nuns interrupted their isolation in the convent; 38 had a career in The Netherlands as a nursery or primary school teacher or a hospital nurse, 22 had a career as a missionary in the tropics, and 6 had a career in both The Netherlands and the tropics. The missionaries had worked in Asia, Africa, and South America. By the start of the outbreak, the convent had become a nursing home for the 9 nuns who never left and for the 66 career nuns who had retired into the convent. The nuns had daily common social and religious activities and were supported by 24 staff members living outside the convent.

Since 1922, no other outbreak of coughing had been reported by the nuns or in the convent archives. At the start of the epidemic, the mean age of the nuns was 75 years (range, 59–91 years), and that of the staff was 26 years (range, 21–47 years). The incidence of pertussis was higher among the 75 nuns in the convent (45 of 75; 60%) than among the 24 staff members living outside the convent

Table 1. Relationship between pertussis incidence, age and duration of isolation from society among the 75 nuns with (n=66) and without (n=9) a career outside the convent.

	Pertussis incidence			Duration of cough among nuns with pertussis		
Group	No. with pertussis/ total	Percentage	*P* for linear trend in proportions	Median days (range)	*P* for trend on rank	No. of death among nuns with pertussis
Age group, years			.31		.20	
55-64	8/16[a]	50		41[b] (28-98)		1
65-74	13/21	62		67 (14-268)		0
75-84	16/27	59		51 (11-178)		0
85-94	8/11[a]	73		83[b] (28-236)		3
Duration of isolation from society,[c] years			.005		.72	
0-6	5/15	33		55 (28-173)		1
7-13	9/17	53		38 (14-268)		0
14-20	10/16	63		88 (11-178)		1
21-34	9/12	75		45 (30-236)		1
35-70	12/15	80		53 (14-159)		1

[a] No significant difference between the incidence of pertussis in the youngest and oldest age group (*P* = .21, Fisher's exact)

[b] No significant difference between the median duration of cough in the youngest and oldest age group (*P* = .10)

[c] Duration of isolation of the 9 nuns without a career outside the convent and the duration of isolation since retirement in the 66 nuns with a career outside the convent

(2 of 24; 8%) ($P = .0003$). All 75 nuns and 5 of the 24 staff members were not vaccinated against pertussis. One vaccinated and one unvaccinated staff member had pertussis. The duration of cough among nuns with pertussis was 11–268 days (mean duration, 55 days). Four of the nuns had pertussis when they died. The pertussis incidence was higher among nuns with a lifelong career in the convent ($n = 9$) than among nuns with a career outside the convent ($n = 66$) (100% vs. 55%, respectively; $P = .007$).The pertussis incidence among nuns who had worked in Dutch society only ($n = 38$) was 74%; in those who had worked in the tropics only ($n = 22$), it was 35%; and in those who had worked in both the Dutch society and the tropics ($n = 6$), it was 17% (χ^2 for linear trend in proportions, $\geq$19.8; $P < .0001$).

The pertussis incidence among all 75 nuns was not statistically significantly related to age ($P = .31$) (table 1). Pertussis incidence also did not increase with increasing total duration of isolation (before and after a career outside the convent) ($P = .08$), but it did increase with increasing duration of isolation after a career outside the convent ($P = .005$) (table 1). In the logistic regression model, duration of isolation after a career outside the convent remained associated with pertussis incidence ($P = .01$) after adjustment for age. Severity of illness, expressed as duration of cough, was not associated with age group or duration of isolation. Three of 4 deaths among nuns with pertussis were in the oldest age group (85–94 years) (table 1).

Discussion

The risk of pertussis in nuns during the pertussis outbreak in this convent was associated with duration of isolation from society and was independent of age. A career outside the convent protected against pertussis. A stay of up to 70 years in the convent—partly interrupted by careers in The Netherlands (an area with lower pertussis endemicity) and/or the tropics (an area with higher pertussis endemicity)—followed by a pertussis outbreak can be seen as a natural experiment.

We assume that social isolation in the convent protected against immunity through natural *Bordetella pertussis* infection and explain the epidemic as being the result of an increase in susceptibility once *Bordetella pertussis* was introduced into the convent. The nuns were never vaccinated against pertussis as children, and, if they had been vaccinated, the effect of the vaccination would have waned.

Indeed, the 1992 epidemic was the first reported in the history of the convent, according to the well kept archive of the convent and the long-term common memory of the nuns. Moreover, the epidemic occurred after the convent had changed from a closed congregation into an open nursing facility for elderly nuns aided by civil staff. Also, the 100% pertussis incidence among the 9 nuns isolated since their entry into the convent and the overall pertussis incidence of 60% (with a case-fatality rate of 9%) indicate a first epidemic.

It could be argued that, in this epidemic, the nuns experienced the first cases of pertussis in their lives. This is unlikely, because the nuns entered the convent at 18–34 years of age when infections were prevalent and everyone experienced pertussis before the age of 15 years.[10] It is estimated that infection-acquired immunity wanes after 4–20 years and that vaccine-acquired immunity wanes after 4–12 years.[11]

Some nuns developed serious pertussis with a high mortality rate, comparable to pertussis in very young children without completed immunization. Natural infection with *Bordetella pertussis* contracted in society effectively protected other nuns against pertussis. Indeed, in The Netherlands in 1995–1996, the estimated incidence of infection was 6.6% per year among individuals 3–79 years of age, up to 10.8% among individuals 20–24 years of age, and higher than the incidence of notified cases (.01%).[12] However, both infection-acquired immunity and vaccine-acquired immunity wane over time. We conclude that booster doses of pertussis vaccines may be a valuable strategy to control pertussis in populations with national childhood vaccination programs.

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Part 2. Other infectious diseases

chapter

6

An epidemic of *Salmonella typhimurium* associated with traditionally salted, smoked and dried pork ham

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Abstract

Objective. To discover the cause of an outbreak of gastro-enteritis after a family party.

Design. Retrospective cohort study.

Methods. All 109 party-goers were asked to complete a written questionnaire about consumed food products and demographic and clinical variables and to hand in a faeces sample. The data were collected at the Public Health service Midden-Limburg, The Netherlands. Faeces and the remaining food products were examined microbiologically. The attack rates and the incidence rates of positive faeces culture among consumers and non-consumers of specific food products were calculated as well as the corresponding relative risks (RR) with 95% confidence intervals (95% CI).

Results. The overall attack rate was 35%. *Salmonella typhimurium* phage type 20 was found in Coburger ham and statistically significantly more frequently in faeces of ill compared with non-ill party-goers (RR: 6.4; 95% CI: 2.5-16.1). Twenty-eight different food products were served. Consumption of Coburger ham only, was statistically significantly related to a positive faeces culture (RR: 4.1; 95% CI: 2.0-8.5). Only consumption of Coburger ham and of bone ham was statistically significantly related to being ill (RR: 2.4; 95% CI: 1.5-4.0 and RR: 1.4; 95% CI: 1.1-1.9, respectively). Coburger ham and bone ham originated from the same batch of raw meat and were prepared in the same manner in the same salt bath. The shorter duration of salting and drying of Coburger ham compared with bone ham corresponded with a higher relative risk of becoming ill.

Conclusion. Consumption of Coburger ham and bone ham infected with *Salmonella typhimurium* phage type 20 caused the outbreak. Traditional salting, drying and smoking of raw pork meat was not antimicrobiologically effective against *Salmonella typhimurium*. Investigation of the antimicrobiological effect of the traditional preparation of meat and the importance of Good Manufacturing Practices and quality control in all stages of production of pork meat, according to the principles of Hazard Analysis and Critical Control Points, is advised.

Introduction

In the Netherlands, a country with a population of 15.5 million Nederland (1996), annually some 7 million episodes of acute gastroenteritis occur, leading to 2.3 million General Practitioner consultations, 3.6 million episodes of drug treatment for an average of 2 weeks, and 12,000 years of school or work absenteeism.[1,2] Essential to all efforts on collective prevention is a knowledge of sources of gastroenteritis. Thus viewed, it is advisable to track down the agent, source and mode of transmission of outbreaks, so that preventive procedures can be adjusted, where necessary. These considerations led us to investigate the cause of an outbreak of gastroenteritis.
The immediate grounds for the investigation were a report from a nursing home employee to the Public Health Service (PHS) of Midden-Limburg in March 1998, to the effect that of the 109 individuals attending a family party, a large number was nauseated and had diarrhoea the next day.

Patients and methods

Four days after the party, the PHS instituted a retrospective cohort study among all 109 partygoers, which included the individual who had reported the incident. Two meals had been served at the party: a selection of confectioneries and a buffet. Symptoms and the foods consumed were tabulated until the end of the outbreak with the aid of structured personally addressed questionnaires in writing.

Ten to 14 days after the party, faecal samples were collected and cultured for *Salmonella*, *Shigell*a, *Campylobacter jejuni* and *Yersinia enterocolitica* according to standard bacteriological methods (Streeklaboratorium, Atrium Medisch Centrum, Heerlen). Confirmation of the culture results and sero- and phage typing was performed according to 'standard operating procedure' number M501 [Diagnostic Laboratory for Infectious Diseases and Screening, Special Bacterial Typing, National Institute for Public Health and the Environment (RIVM), Bilthoven].[3,4]
Leftovers from one of the food products consumed was used to perform an aerobic colony count for *Bacillus cereus*, *Campylobacter, Clostridium perfringens*, *Enterobacteriaceae*, *Salmonella*, *Staphylococcus aureus*, and yeast and moulds by the Inspectorate for Health Protection/Food Inspection Service (IGB/KvW), Maastricht.
The food preparation process and location at which preparation took place were examined using standard methods (IGB/KvW), Maastricht. The results of the

microbiological tests came in and were made known to the partygoers after all completed questionnaires had been returned.

Definitions and statistics

Partygoers were categorized as 'ill' if they succumbed to nausea, vomiting, stomach ache, diarrhoea, chills or fever within a week after the party. The median latency period time was calculated, as were the attack rates of becoming ill and the incidence rates of positive faecal specimens amongst consumers and non-consumers of specific food products.
The corresponding relative risks were calculated with 95% confidence intervals (95% CI), using the computer program SPSS (Statistical Package Social Sciences International BV, Gorinchem).

Results

Of the 109 partygoers, 86 (79%) handed in a completed questionnaire. The average age of this study group, consisting of 42 females and 44 males was 43 years (range: 13-77). The overall attack rate was 35% (30/86) and the median incubation time 12 hours. The average age of the ill individuals and those who remained healthy was respectively 41 years (13-69) and 44 years (21-77). Ill individuals reported nausea (53%), vomiting (3%) stomach ache (90%), diarrhoea (93%), chills (83%), and fever (63%). The average duration of the illness was 6 days (range: 1-21).
Table 1 shows the attack rates and RRs with 95% CI among both consumers and non-consumers of the 28 food products. A statistically significant RR for illness was found only for the products Coburger ham and bone ham, namely 2.4 (1.5-4.0) and 1.4 (1.1-1.9) respectively.

Faecal specimens were collected from 78 (91%) persons from the study group. Of the 30 ill individuals, 1, and of the 56 healthy subjects, 7 did not submit a faecal sample (no statistically relevant difference). The culture was positive for *Salmonella typhimurium* phage type 20 in 33 (42%) individuals (table 2). Of the 33 subjects with a positive culture, 79% had become ill, in contrast to the 9% of the 45 individuals with a negative culture who fell ill; a statistically significant difference (RR: 6.4; 95% CI: 2.5-16.1). *Shigella*, *Campylobacter jejuni* and *Yersinia enterocolitica* were not cultured.

Table 1. Food products consumed at a party, subsequent to which the partygoers became ill; the relative risks of illness among 86 consumers and non-consumers of 28 food products are shown.

Food Product	% Ill (number ill/total number)[a]		Relative Risk (95% CI[b])
	Consumers	Non-consumers	
Confectioneries			
- pineapple bavarois	0 (0/6)	37 (29/78)	0.6 (0.5-0.7)
- apricot special	29 (2/7)	35 (27/77)	0.9 (0.5-1.5)
- Christopher	33 (2/6)	35 (27/78)	1.0 (0.5-1.8)
- apricot	44 (4/9)	33 (25/75)	1.2 (0.6-2.2)
- apple-lemon	56 (5/9)	32 (24/75)	1.5 (0.7-3.2)
- rice with cream	56 (5/9)	32 (24/75)	1.5 (0.7-3.2)
- crumble	71 (5/7)	31 (24/77)	2.4 (0.7-7.8)
- hazelnut bavarois	71 (5/7)	31 (24/77)	2.4 (0.7-7.8)
Buffet			
- sliced dried sausage	0 (0/4)	35 (27/76)	0.6 (0.5-0.8)
- cold dish	30 (20/66)	50 (10/20)	0.7 (0.4-1.1)
- beef in sauce	30 (11/37)	36 (16/45)	0.9 (0.7-1.2)
- stuffed tomatoes	31 (9/29)	37 (21/58)	0.9 (0.7-1.2)
- mini rolls	33 (8/24)	34 (23/61)	1.0 (0.7-1.4)
- fruit	36 (10/28)	34 (19/55)	1.0 (0.7-1.4)
- Italian salad	36 (5/14)	32 (18/57)	1.1 (0.7-1.6)
- asparagus with ham	37 (19/51)	32 (11/34)	1.1 (0.8-1.5)
- pork cutlet in cream sauce	39 (11/28)	33 (19/57)	1.1 (0.8-1.6)
- devilled eggs	38 (22/58)	30 (8/27)	1.1 (0.5-2.3)
- sliced chicken ham	40 (2/5)	32 (24/74)	1.1 (0.5-2.3)
- French bread	41 (18/44)	30 (14/40)	1.2 (0.9-1.6)
- fresh fruit	43 (9/21)	30 (18/59)	1.2 (0.8-1.8)
- Leudal salad	41 (9/22)	28 (12/45)	1.2 (0.8-1.8)
- salmon	40 (19/47)	26 (9/35)	1.2 (0.9-1.7)
- chicken salad	42 (8/19)	27 (15/55)	1.3 (0.8-1.9)
- cocktail sauce	48 (14/29)	27 (14/51)	1.4 (0.9-1.2)
- roast beef with pepper sauce	54 (6/11)	31 (22/70)	1.5 (0.8-2.9)
- bone ham	43 (23/54)	18 (5/28)	1.4 (1.1-1.9)
- Coburger ham	66 (21/32)	16 (8/51)	2.4 (1.5-4.0)

[a] The total of consumers plus non-consumers per food product is not always 86 as not all questionnaires were completely filled in

[b] 95% CI=95% confidence interval

The percentages of stool cultures that were positive for *Salmonella typhimurium* phage type 20 among consumers and non-consumers of the 28 food products were calculated. Only Coburger ham demonstrated a statistically significant relation with the *Salmonella typhimurium* positive stool culture (RR: 4.1; 95% CI: 2.0-8.5). In the group of 78 individuals who submitted both a completed questionnaire and a faecal specimen, consumption of the combination Coburger ham

Table 2. The distribution of 78 subjects with a positive or negative stool culture for Salmonella typhimurium (phage type 20) by number of individuals who became ill/not ill.

Culture Results	Ill	Not Ill	Total	Relative Risk (95% CI[a])
Positive	26	7	33	
Negative	4	41	45	
Total	30	48	78	6.4 (2.5-16.1)[b]

[a] 95% CI = 95% confidence interval

[b] Relative risk for illness with a positive culture result compared to a negative culture result

and bone ham explained 31 of the 33 positive faecal specimens; this combination yielded the greatest chance of a positive faecal sample, compared to all other combinations eaten. (RR: 2.0; 95% CI: 1.5-2.6).

A remaining piece of the batch of Coburger ham served at the party was recovered from the butcher's, in which piece *Salmonella typhimurium* phage type 20 was detected.

No bone ham or other food products were further available for microbiological examination. The Coburger ham and the bone ham were prepared from the same batch of raw pork meat, supplied by the same slaughterhouse. They were prepared by the butcher, who was compliant with all hygiene and building requirements, in the same brine solution according to traditional methods. The Coburger ham and the bone ham were prepared by cooling the raw butt with bacon and, respectively, the entire ham (12 h at 1ºC), followed by immersion in brine for 3 respectively 4 weeks (24% sodium nitrite, 0.5% glucose, 0.25% potassium nitrate and 75.25% water; brine strength: 22-24º on the Baumé scale, a scale used to express the concentration of solutions) using a ratio of meat: brine = 1:2, then salting outside the brine (10 days at 6ºC in the dark), rinsing (2 h in cold running water), drying (1 h at 50ºC), cold smoking in a drying room (for respectively 14 days and 2 months at 15-20ºC).

Discussion

Our investigation led us to conclude that the outbreak was caused by the consumption of bone ham and Coburger ham contaminated by *Salmonella typhimurium* phage type 20.

In view of the severity and duration of the symptoms and the average low age of the individuals who became ill, the infective dose was probably high. Selection distortion was considered unlikely as 79% of the partygoers completed the questionnaire, of whom 91% took part in the stool examination, in which no significant difference was seen in percentage of individuals who were ill and those who were not. For this reason, no non-response study was conducted, all the more as the partygoers were informed of the results of the microbiological test after returning the questionnaires.

We also concluded that the Coburger ham and bone ham were the original source of *Salmonella typhimurium* at the party. Cross-contamination from the other food products served appeared to be ruled out. After all, *Salmonella typhimurium* phage type 20 was also detected in the remaining piece of the Coburger ham found at the butcher's. Moreover, the other food products showed no statistically significant relation with either a positive faecal culture or with becoming ill. Furthermore, of all the combinations of foods eaten, consumption of the combination of Coburger ham and bone ham explained the greatest number of positive stool cultures.

Cross-contamination of Coburger ham and bone ham by other raw meat products at the butcher's does not appear likely. After all, the butcher was compliant with all Dutch hygienic requirements and *Salmonella typhimurium* was found, not only on, but also in, the Coburger ham. Also, the Coburger ham and the bone ham had been made from the same batch of raw pork meat in the same brine solution. And the higher RR for becoming ill from the Coburger ham compared to the bone ham fits with the fact that Coburger ham is brined and dried for a shorter period than bone ham. We moreover established that traditional salting, smoking and drying of raw pork meat was not antimicrobiologically effective against *Salmonella typhimurium* phage type 20, occurring in the batch of raw pork meat supplied to the butcher, from which the Coburger ham and bone ham were subsequently prepared.

The literature supports these conclusions. *Salmonella* is frequently found in the alimentary canal of the pig, and in many cases – 71% - *Salmonella typhimurium* is the type involved.[5] During the slaughtering process in Dutch slaughterhouses, 30% of all carcasses are contaminated via the content of the gut with *Salmonella* strains, which ultimately leads to contamination of the raw meat.[6,7] In 1997, the main strain isolated from 14% of the raw ground pork meat and from 8% of

all raw pork meat examined in the Netherlands was *Salmonella typhimurium*.[8] The literature also notes that bacteria can withstand salt concentrations of up to 4.5 M NaCl, that the antimicrobiological effect of nitrate and nitrite is limited and that bacteria can continue to grow in dried, salted ham for at least three months.[9-12]
Annually, some 3% of the Dutch population is infected mainly with *Salmonella typhimurium* and *Salmonella enteritidis,* which results in 400,000 episodes of gastroenteritis.[1,13]
Since 1985, pigs have been the leading source of infection with *Salmonella typhimurium* (W. van Pelt, Centre for Infectious Diseases Epidemiology, Diagnostics and Screening for Infectious Diseases, RIVM, written bulletin, 1998).[5]

Conclusion

In the light of the results of our study, we advise further investigation of whether traditional salting, drying and smoking of raw pork meat is sufficiently effective against *Salmonella* strains in raw pork meat, all the more as no data proved to be available in the literature on the subject. Pork meat products that undergo a less rigorous salting, smoking and drying process, such as bacon, salt pork, Canadian bacon, slab bacon, bacon square, Lachsschinken, casselerrib and pickled pork, are all products qualifying in this respect.[14]
This study underscores the importance of good manufacturing practice (GMP) and quality control, according to the principles of Hazard Analysis and Critical Control Points (HACCP), in all stages of production of pork meat.[15]

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chapter

7

A gastroenteritis epidemic caused by a Norwalk-like virus after two weddings in a restaurant; a plea for integral microbiological investigation

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Abstract

Objective. To elucidate the cause of an epidemic of gastroenteritis amongst the guests and waiting staff of two weddings celebrated on the same day in the same restaurant.

Design. Retrospective and descriptive.

Method. Following an outbreak of gastroenteritis amongst 215 wedding guests and restaurant staff in 1999, the Public Health Institute Midden-Limburg, the Netherlands, inventoried the demographic and clinical variables and consumed foods by means of a structured written interview. Faecal samples and remaining food products were bacteriologically examined, and later on faeces were virologically examined for the presence of the Norwalk-like virus (NLV). The attack and the incidence rates of NLV-positive faecal specimens amongst consumers and non-consumers of specific food products were calculated, as well as the corresponding relative risks with 95% confidence intervals.

Results. The overall attack rate was 66%. A NLV with the same genotype was found statistically significantly more frequently in faecal specimens of ill persons compared with non-ill individuals. Of the 61 different dishes served, two showed a statistically significant relation with being ill. These food products were eaten by 26% of the ill persons. No statistically significant association was found between NLV-positive faeces and consumption of a specific dish. The index case began having symptoms of gastroenteritis at the morning of the wedding party, before food was served. The guests of the two wedding parties used the same entrance and toilets.

Conclusion. The epidemic was caused by a single NLV strain. No association could be found between the consumption of certain food products and being ill. The NLV epidemic was probably caused through direct or indirect contact between wedding guests and restaurant personnel. In the case of such reports of gastroenteritis epidemics, it is strongly recommended to test faecal, food product and environment samples for both bacteria and viruses, with an emphasis on NLV, to ensure early diagnosis.

Introduction

In the Netherlands, a country with a population of 16 million (2000), annually some 7 million episodes of acute gastroenteritis occur, leading to 2.3 million GP consultations, 3.6 million episodes of drug treatment for an average of 2 weeks, and 12,000 years of work or school absenteeism.[1,2] A recent prospective population cohort study showed a standardised incidence of gastroenteritis of 283 per 1000 person years, with bacteria accounting for 5% of the incidence, bacterial toxins for 9%, parasites for 6% and viruses for 21%; in 11 of the latter mentioned, the culprits are Norwalk-like caliciviruses (NLVs).[3] Recent studies have moreover revealed NLVs as the leading cause of all epidemics of gastroenteritis reported to the Dutch municipal public health services, accounting for fully 87% of cases.[4] The exact contribution of NLV as cause of food-related outbreaks in the Netherlands is unknown. Studies in Japan, United Kingdom and the United States indicate that their contribution is considerable.[5]

Essential to efforts on collective prevention is a knowledge of sources of gastroenteritides. Active surveillance of gastroenteritis epidemics can be used to establish the microbiological cause, source, transmission and spread.[6] This would allow the direct implementation of intervention measures and the adjustment, where necessary, of procedures aimed at prevention. If persons working in health care or the food industry are involved in outbreaks of gastroenteritis, the risk of infection for their patients or products respectively constitutes an additional reason for active surveillance (see the scenario for 'Outbreaks of gastroenteretis in institutions', compiled by the National Coordination of Infectious Disease Control (1996); www.infectieziekten.info).[7]

These considerations led us to investigate the cause of an outbreak of gastroenteritis. The immediate ground for the investigation was a report from a nursing home employee to the Public Health Service (PHS) of Midden-Limburg in August 1999, that after her wedding, numerous guests had become ill with symptoms of nausea, vomiting and diarrhoea.

Patients and Methods

Circumstances

The bride's report stated that the wedding had been celebrated in a restaurant where a dinner and a supper were served. The bride had invited co-workers

from the nursing home. The situation was mapped out in consultation with the restaurant manager. The restaurant had one kitchen and on the day of the wedding, three other weddings were also celebrated with respectively a supper, a dinner and a dinner which was later followed by cocktail snacks. A fifth group held a barbecue in the evening. Guests at the second wedding and staff that waited at both wedding 1 and 2 developed gastroenteritis. The staff in question had also eaten several of the dishes served at weddings 1 and 2. Inquiries revealed that none of the guests at wedding 3 and 4, or at the barbecue fell ill. The meals served to all 5 groups of guests were partially made up of the same dishes. The guests at weddings 1 and 2 made use of the same entrance and sanitary facilities. These were separate from the guests at weddings 3 and 4 and from the barbecue group, who all used a different entrance and set of sanitary facilities, and other rooms.

Retrospective cohort study

On the day the report was made, four days after the wedding, the PHS instigated a retrospective cohort study among the guests attending wedding 1 (n = 157) and wedding 2 (n = 47) and among the restaurant staff. Symptoms were recorded until the end of the outbreak; with the aid of a structured questionnaire personally addressed to each, all the dishes partaken from were tabulated, in order to detect the source of the pathogen.

Preventive measures

As laid down in the protocol ('Outbreaks of gastroenteritis in institutions', compiled by the National Coordination of Infectious Disease Control (1996); www.infectieziekten.info), all restaurant staff and the wedding guests employed in a nursing home and whose work involved either the preparation, packaging or treatment of food and beverages or nursing activities, were asked not to work in the institutional kitchen or to perform nursing activities until their symptoms had disappeared and any positive bacterial culture results came back negative.

Stool tests

Faecal samples were collected both from subjects who had become ill and from those who had not, 5 to 10 days after the wedding; these samples were cultured first for *Salmonella, Shigella, Campylobacter jejuni* and *Yersinia enterocolitica* according to the standard methods used at the Regional Laboratory, Atrium Medisch Centrum, Heerlen. After the bacterial culture results came in, 78 faecal samples were tested for the presence of NLV at the Laboratory for Infectious

Diseases, National Institute for Public Health and the Environment, using 'reverse transcription'-PCR (RT-PCR).[4] NLV positive RT-PCR products subsequently underwent sequential analysis and the genotype of the virus was determined using phylogenetic analysis. [4]

Food testing

Leftovers from the food products consumed during the festive meals were examined microscopically with acridine orange stain and gram stain, and an aerobic colony count was performed for *Bacillus cereus, Clostridium perfringens, Enterobacteriaceae, Salmonella, Staphylococcus aureus* and yeast and moulds by the Inspectorate for Health Protection and Veterinary Public Health in The Hague. The food preparation process and location at which preparation took place were examined using standard methods by the same Inspectorate.

At the time of this outbreak, the Netherlands did not yet have a microbiological laboratory where food products could be examined for the presence of NLV.

Definitions and statistics

Persons were categorized as 'ill' if they succumbed to nausea, vomiting, stomach ache, diarrhoea, chills or fever within a week after the gathering. The median incubation time was calculated from the start of the wedding.
Attack rates and incidence rates of positive faecal specimens amongst consumers and non-consumers of specific food products were calculated.
To achieve the largest possible groups, guests and staff were combined into 4 risk groups, all of whom had consumed a different combination of dishes.[8]
The corresponding relative risks with 95% confidence intervals were calculated with the χ^2 test.
All calculations were performed using the computer program 'Statistical Package for the Social Sciences' (SPSS, International BV, Gorinchem) and EpiInfo, version 6.02 (EpiInfo 6, Centers for Disease Control, Atlanta, VS).

Results

Attack rates

Of the guests attending weddings 1 and 2 and of the restaurant staff, respectively 70% (110/157), 77% (36/47) and 82% (9/11) completed the questionnaire, a total response of 72% (155/215). The average age of this more closely studied group of 155 persons, composed of 86 females and 69 males, was 37 years (range:

2-91). The overall attack rate in the study group was 66% (102/155); among the guests at weddings 1 and 2 and among the restaurant staff respectively, the specific attack rate was 70% (77/110), 50% (18/36) and 78% (7/9) (Table). The average age of the subjects who had become ill was 38 years (range: 5-91); among those who had not become ill this was 33 years (range: 2-64). Ill individuals reported nausea (n = 81; 79%), stomach ache (n = 72; 71%), diarrhoea (n = 65; 64%), vomiting (n = 55; 54%), headache (n = 51; 50%), chills (n = 42; 41%), fever (n = 35; 34%) and muscle pain (n = 33; 32%). The average duration of the illness was 50 h (range: 12-72). None of the ill individuals were hospitalized. The median incubation time was 40 h. Two people fell ill on the day of the wedding. The majority became ill on the second day after the wedding, including the first victims among the personnel of the restaurant (Figure).

NLV infection

Faecal specimens were obtained from 59 of the 102 ill individuals, and from 19 of those who did not become ill. The 78 stool cultures were negative for *Salmonella, Shigella, Campylobacter. jejuni* and *Yersinia enterocolitica*. NLV-RNA was detected in 40 of the 59 faecal specimens from the subjects who were ill and in 4 of the 19

Table. Risk of illness following exposure to Norwalk-like virus (NLV) (estimate based on a positive faecal culture) among guests at 2 weddings and staff of the restaurant at which the guests consumed a meal.

Groups studied	Number	Ill persons (%)	Faecal culture	
			NLV positive (%)	Ill persons (%)
guests wedding 1	110	77 (70)	35/62 (56)	33/35 (94)
guests wedding 2	36	18 (50)	6/11 (55)	4/6 (67)
personnel	9	7 (78)	3/5 (60)	3/3 (100)
total group	155	102 (66)	44/78 (56)	40/44 (91)
Risk groups‡				
risk group 1	34	27 (79)	16/17 (94)	14/16 (88)
risk group 2	91	69 (76)	30/52 (58)	29/30 (97)
risk group 3	36	18 (50)	6/11 (55)	4/6 (67)
risk group 4	127	87 (69)	36/63 (57)	33/36 (92)

*Value of *P* on assessment of the difference in the percentage of ill individuals with a positive faecal culture and the percentage of ill individuals with a negative faecal culture (X2-test)
†Statistically significant
‡At wedding 1, 34 guests and staff (risk group 1) partook of 17 dishes at the dinner and 91 guests and staff (risk group 2) of 37 dishes at supper. At wedding 2, 36 guests and staff (risk group 3) partook of 26 dishes at the supper. Risk group 4 was formed of the 127 guests and staff who had partaken at both suppers

faecal specimens from the healthy group (RR: 1.63; 95%-CI: 1.19-2.22; Table), a statistically significant difference. All NLV strains had the same genotype (GI, Southampton).

At wedding 1, 34 guests and restaurant staff (risk group 1) partook of 17 dishes at the dinner, while 91 guests and restaurant staff (risk group 2) consumed 37 dishes at the supper. At wedding 2, 36 guests and restaurant staff (risk group 3) partook of 26 dishes at the supper. Risk group 4 was made up of the 127 guests and restaurant staff partaking of 18 dishes from both suppers. The attack rate in the risk groups varied from 50-79% (Table). The index case belonged only to risk group 1. She was nauseated and vomited on the morning of the wedding. She did not submit a faecal sample.

Risk of illness associated with specific dishes.

To assess whether the consumption of specific dishes was associated with falling ill, dish-specific attack rates were calculated in the 4 risk group. A statistically significant relation was found between 'consommé' and becoming ill in risk group 1 (RR: 1.66; 95%-CI: 1.04-2.65) and in risk group 3, between the 'grilled chicken breast filet' and becoming ill (RR: 2.79; 95%-CI: 1.45-5.37). No suspi-

Ill persons (%) among those with an NLV negative faecal culture	Relative risk (95%-CI)	*P**
15/27 (56)	1.41 (1.07-1.87)	0.005†
3/5 (60)	1.1 (0.45-2.77)	0.82
1/2 (50)	2.00 (0.50-8.00)	0.17
19/34 (56)	1.63 (1.19-2.22)	0.0003†
0/1 (0)		0.03†
15/22 (68)	1.42 (1.06-1.90)	0.005†
3/5 (60)	1.11 (0.45-2.77)	0.82
18/27 (67)	1.38 (1.03-1.83)	0.01†

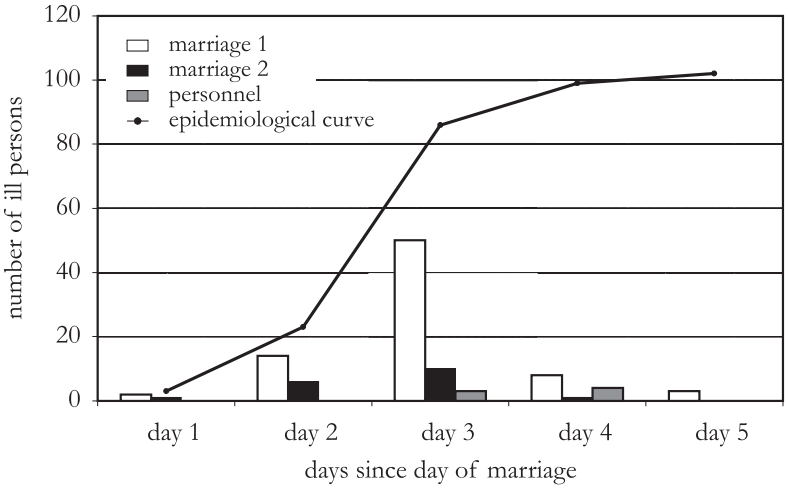

Figure. The cases of illness and cumulating of cases (shown as an epidemiological curve) among the guests at the 2 weddings and among the staff of the restaurant at which the guests consumed a meal; day 1 is the day of the wedding.

cious dishes were found in risk groups 2 and 4. Of the 102 ill subjects, 27 (26%) had partaken of the 2 suspicious dishes.

Dishes as source of NLV?

To identify certain dishes as a source of NLV, dish-specific infection percentages were calculated in the 4 risk groups. The faeces of 92% (11/12) of those consuming the suspicious dishes and the faeces of 67% (10/15) of individuals, who had not consumed the 2 suspicious dishes, were NLV positive. This is not a statistically significant difference (RR: 1.38; 95%-CI: 0.92-2.04). None of the other dishes consumed showed any statistically significant relation with NLV positive faeces.

Thirteen leftover food products, sauces and ingredients from the wedding meals were collected from the restaurant and examined. None of these products showed any trace of *Bacillus. cereus*, *Campylobacter, Campylobacter perfringens, Enterobacteriaceae, Salmonella, Staphylococcus aureus*, yeasts or moulds. The restaurant was in compliance with the hygienic and architectural legal requirements and operated in accordance with the principles of 'Hazard Analysis and Critical Control Points' (HACCP).[9]

Discussion

We may conclude that an outbreak of gastroenteritis occurred among the guests at 2 weddings and the serving staff, caused by an identical strain of Norwalk-like virus, which in all likelihood was transmitted not by food, but by person-to-person contact and contact with contaminated surfaces.

Epidemic gastroenteritis caused by NLV

NLVs are the main agents to cause epidemic gastroenteritis in humans.[10] The prototype of this group of caliciviruses, the Norwalk virus, was discovered in 1972 in the faeces of volunteers who were fed bacteria-free faecal filtrates derived from schoolchildren in the town of Norwalk (Ohio, US), who had been felled by an outbreak of acute gastroenteritis.[11,12] Vomiting is characteristic for NLV infections; enabling the virus to spread via vomit and aerosol droplets of vomit. NLV infections are generally mild in children, but for most adults, the onset of symptoms is extremely acute, usually accompanied by projectile vomiting and diarrhoea.

The infective dose is as small as 10-100 virus particles. Uncooked food such as raw oysters, cockles and mussels, and food handlers contaminated with NLV are regularly implicated sources of infection. The virus spreads via the faecal-oral route, transmitted in contaminated water and food, although the usual route is person-to-person contact.[12]

Food unlikely the source of NLV in this case

The high rate of participation in our study survey, renders the outcome of the investigation into whether the NLV outbreak was foodborne or not, very reliable. While no non-response study was performed, and a relatively higher percentage of ill individuals submitted faecal samples than individuals who remained healthy, it is unlikely that distortion occurred at the level of suspicious exposure factors, as the results of the study were published after the questionnaires were handed in. Another argument against a foodborne outbreak is the fact that the kitchen was in compliance with HACPP standards and that no uncooked food, raw fish products or oysters were served. We view the fact that 26% of those who became ill had partaken of 2 statistically suspicious dishes as a coincidence. After all, a large number – 61 in total - of different dishes was examined. Moreover, 1 of the 2 suspicious dishes was also served at weddings 3 and 4, where no one became ill.

Transmission of NLV through direct contact

This outbreak may also have been caused by NLV transmission via direct or indirect contact between wedding guests and personnel. An argument in favour of a person-to-person outbreak is the fact that one person became ill on the day of the wedding itself, even before the food was served: the index person. As a key player in risk group 1, she participated in greeting the family before and during the dinner. The people in risk group 1 subsequently became part of risk group 2 during the rest of the day, which group came into contact with risk group 3 in the shared hall and sanitary facilities. This fits well with the decreasing attack rates in risk groups 1, 2 and 3: respectively 79%, 76% and 50%. As a wedding guest was the first to succumb, with restaurant staff becoming ill only after 2 days following the wedding, contamination via a member of the serving staff appears unlikely.

Also, the NLV outbreak described by us was characterized by vomiting in more than 50% of the ill individuals, an incubation time of between 24-28 h and illness duration of between 12-60 h.[12] These characteristics led to the viral diagnosis following analysis of the questionnaires.

The fact that no virus was found in the faeces of 32% of those becoming ill may have resulted from a disease definition that was simply not specific enough, but is more likely due to the tardy collection of faecal samples (5–10 days after the weddings). NLV excretion reaches maximum levels during the acute phase (24-72 h), although excretion up to minimally 10 days has been observed in volunteers. As NLV levels in the stool are usually low, the best chance of actually diagnosing NLV infection is by ensuring that an outbreak of gastroenteritis is reported without delay, directly followed by the collection of faeces from symptomatic individuals.

Had bacterial and viral testing been performed simultaneously during this outbreak, the viral diagnosis would have probably been determined earlier. The victims and restaurant owner would have been informed earlier, in particular about the consequences for the prevention policy to be followed. After all, recovered food workers and nursing staff with a negative bacterial culture are permitted to return to work ('Outbreaks of gastroenteritides in institutions', compiled by the National Coordination of Infectious Disease Control (1996); www.infectieziekten.info), whereas persons with a positive viral culture may not, in the light of the chance of viral shedding, which may last for days, and transmission of the low infective dose of NLV (Protocol 'Calicivirus' (2002, National Coordination of Infectious Disease Control (1996); www.infectieziekten.info). Workers may

return to work according to this protocol 24-72 hours after these symptoms have indeed gone and stringent adherence to hand and toilet hygiene is enforced (Protocol 'Calicivirus' (2002).

Conclusion

On the grounds of this study we strongly advocate performing bacterial and viral tests simultaneously in the event of gastroenteritis, especially if persons working in the food industry or in nursing are involved. We advocate this in part because of the high prevalence of NLV in epidemics of gastroenteritis in the Netherlands,[3,13] the lack of insight into the prevalence of the symptoms during a gastroenteritis epidemic prior to analysis of the questionnaires and the low predictive value of symptoms regarding the cause of an outbreak.[14]
In addition, viral examination of the surroundings and mandatory saving of leftover food samples from the restaurants and central kitchens may not be absent, enabling an implicated food product to be easily taken out of circulation.

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chapter

8

Risk of introduction of poliovirus into a Dutch Cape Verdian community during an outbreak of poliovirus in Cape Verde, 2000

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Summary

Objective. To assess the risk of introduction of polio virus in a Cape Verdian community of Rotterdam, during the polio epidemic in Cape Verde in 2000.

Methods. All 225 insufficiently vaccinated 0–14-year-old children out of all 0–14-year-old Cape Verdian children living in Rotterdam (n = 4188) and a random sample of 285 Cape Verdians out of all 15–30-year-old Cape Verdians (n = 5074) in Rotterdam were surveyed to assess travel behaviour and vaccination coverage. Faecal specimens were collected and sewage samples taken in neighbourhoods with a sizable Cape Verdian population for testing of polio virus.

Results. Of the Cape Verdian children aged 0–14 years living in Rotterdam, 94.6% was sufficiently vaccinated, but 10% of the 91 responding insufficiently vaccinated children aged 0-14 years, reported travelling to Cape Verde during the polio epidemic. Of the responding adults aged 15–30 years living in Rotterdam (n=82), 10% was not vaccinated against polio, and 17% reported travelling to Cape Verde during the polio epidemic.
In the faeces of 80 insufficiently vaccinated individuals aged 0–14 years and in 74 adults aged 15–30 years, no poliovirus was detected.
Samples of sewage from six sites were negative for poliovirus.

Conclusion. No evidence of poliovirus infection was found in the Cape Verdian population in Rotterdam despite extensive travel to the Cape Verde during the outbreak.

Introduction

In the Netherlands, the vaccination coverage with inactivated polio vaccine (IPV) in the national vaccination program is high (97.2% in 2000), but it is not evenly distributed throughout the country.[1] In 2003, 37 (8%) of all 485 municipalities had a coverage rate of < 90% in 9-year-old children; nine of these 37 achieved < 80%.[1] These municipalities are in an area that crosses the country diagonally from the north–east to the south–west, where they surround Rotterdam; they are home to an estimated 275.000 members of Orthodox Reformed religious communities who decline vaccination.[2] These communities have suffered polio epidemics, most recently in 1992–1993,[3] and other vaccine preventable epidemics of measles and rubella.[4,5] Since 1993, the number of new polio-susceptible children has increased by several tens of thousands.[2,6]

The World Health Organization (WHO) is committed to eradicate poliomyelitis.[7] Unfortunately, the campaign has encountered set-backs, with introduction of infection in previously free areas.[8,9] In August 2000, an epidemic of poliomyelitis was reported in Cape Verde, an archipelago in the Atlantic Ocean west of Senegal, where the estimated vaccination coverage in children under 5 years was < 80%.[10]

In November 2000, the WHO warned of the export of wild poliovirus to countries with extensive travel contact with Cape Verde. At this time, neither clinical cases of poliomyelitis nor poliovirus infections had been diagnosed in The Netherlands. Since Rotterdam has a large Cape Verdian community and adequate information was not available about the vaccine coverage of Cape Verdians above the age of 14 years, the possibility could not be excluded of importation of poliovirus into the Cape Verdian community in Rotterdam, from which then transmission might occur into the surrounding unprotected religious communities.

Therefore, the Dutch authorities decided to vaccinate all inadequately (less then three times) vaccinated Cape Verdians aged 0–30 years in The Netherlands with live oral polio vaccine (OPV). To promote the participation in the campaign, anonymous obtainment of OPV was permitted.

The authorities assumed that Cape Verdians above 30 years were naturally protected through the last poliovirus type 1 epidemic in Cape Verde, as reported to them by the WHO to have occurred in 1970.

To assess the risk of introduction of poliovirus into the Rotterdam community and to determine whether the virus was actually introduced before the vaccination campaign, the vaccination coverage, travel frequency to Cape Verde during the polio epidemic and presence of poliovirus in the Cape Verdian community of Rotterdam were studied.

Methods

Study population

In this study, a Cape Verdian was defined as a person with at least with one parent born in Cape Verde, and who was registered in the Municipal Population Register (MPR) of Rotterdam. The target group of the national vaccination campaign against poliomyelitis was divided into two age groups 0–14 and 15–30 years. By cross-referencing the MPR with the Provincial Vaccination Register, all 225 insufficiently vaccinated (< 3 doses IPV) Cape Verdian children from 0 to 14 years were identified among the 4188 that were registered on the MPR. The second study group was a random sample of 285 subjects, obtained from the MPR from all 5074 Cape Verdians aged 15–30 years registered as resident in Rotterdam.

Data collection

A structured questionnaire about travel behaviour to Cape Verde and contact with returned travellers was mailed to both study groups with a request for a faeces sample. The parents of the children < 14 years were also asked about vaccination status of their children and travel to and from Cape Verde after the 1st July 2000, 6 weeks before the first clinical case of poliomyelitis was diagnosed on the islands. The place of residence of all Cape Verdians in Rotterdam was obtained from the MPR. Finally, a non-response analysis was performed to assess if respondents and non-responders aged 0–30 years were evenly distributed throughout the neighbourhoods of Rotterdam, and whether respondents aged 0–14 years were representative of the non-responders aged 0–14 years in frequency of vaccination.

Faeces study

Faeces samples were examined at the National Institute of Health and the Environment for the presence of virus, as recommended by the WHO.[11]

Sewage samples were tested for poliovirus in the six neighbourhoods with the highest number of Cape Verdian immigrants. The locations for sewage sampling were chosen to represent effluent from areas of most dense Cape Verdian congregation. Six sewage samples of 1 l each were taken on a rain-free day to avoid dilution. The samples were collected in sterile bottles kept at 4 0C and transported within 24 h to the laboratory for examination for the presence of poliovirus according to a previously described method.[12]

Statistical analysis

The sample size of group 1 (n = 225) was determined by the total number of the insufficiently vaccinated Cape Verdians in Rotterdam aged 0–14 year. The sample size of group 2 (n = 285) was chosen in such a way that with a participation rate of 25% in the faeces study, a prevalence of infection with polio virus of at least 4% would be detected with a confidence of 95%.

The 95% confidence interval of the prevalence of positive faeces samples was calculated assuming a binomial distribution, because only a positive or negative outcome was possible.[13] The confidence interval in the total group of 0–30-year olds was calculated assuming that if polio virus infection had occurred, it would have been in the insufficiently vaccinated group aged 0–14 years.

Significance was calculated by means of the chi-square test, with Yates' correction (Epi-InfoTM version 5.1; CDC, Atlanta, GA, USA).

Results

The response rate to the questionnaire was 40% among the 225 aged 0–14 years and 29% among the 285 aged 15–30 years. Of the 91 responding 0–14-year-olds with insufficient vaccination against polio, 9 (10%) had visited Cape Verde during the polio epidemic (Table 1) and of these 9 children, three had never received a vaccination against polio (Table 2).

Fourteen (17%) of the 82 responding 15–30-year-olds had had visited Cape Verde during the polio epidemic.

Contact with a person who had been in Cape Verde during the epidemic was frequent, reported in 58% of the group aged 0–14 years with insufficient vaccination and 75% of the 15–30-year-olds.

Table 1. Results of the questionnaire among Cape Verdians in Rotterdam in 2000.

	Group 1	Group 2
Mean age (years)	5	*22*
Male	40 (44)	39 (47)
Ever been to Cape Verde	34 (37)	75 (92)
Visit to Cape Verde during polio epidemic	9 (10)	14 (17)
Contact (any) with person who was on Cape Verde during polio epidemic	58 (64)	62 (75)
Contact at home with person who was on Cape Verde during polio epidemic	50 (55)	56 (68)

Group 1, Cape Verdians 0-14 year less then three times vaccinated (n=91)
Group 2, Cape Verdians 15-30 year random sample (n=82)
Values in parenthesis are percentages

The percentage of Cape Verdians in Rotterdam with sufficient vaccination against polio in the age groups 0–14 years was 94.6% (3963/4188). Of the 225 insufficiently vaccinated 0–14-year-olds, 112 (50%) were vaccinated at least once and 27/225 (24%) were too young to be vaccinated three times (Table 2).

By using the efficacy of polio vaccine after 1, 2 and 3 doses,[14] we calculated that only 150 (3.6%) of all 0–14-year-old Cape Verdian children in Rotterdam were susceptible to infection with poliovirus (calculation shown in Table 2). In the group of 15–30-year-old Cape Verdians and the travellers among them, 90% reported to be vaccinated.

During the observation period, Rotterdam had a population of 592 660, with 14 449 (2.4%) Cape Verdian immigrants who lived in 70 of the 85 city neighbourhoods. However, 39% (5700) of all Cape Verdian immigrants lived in six neighbourhoods from where the sewage samples were taken. The insufficiently vaccinated Cape Verdians were spread proportionately among the Cape Verdian communities.

A faeces sample was provided by 80/225 (35%) of the participants of the group aged 0–14 years and by 74/285 (26%) of the group aged 15–30 years. None of the faecal samples contained poliovirus. Based on the absence of positive findings of poliovirus, it was calculated that with a 95% upper confidence limit, a possible prevalence of poliovirus in the groups aged 0–14 and 15–30 years, and for the total group aged 0–30 years, would not be >3.7%, 4.0% and 1.1% respectively.

Table 2. The observed and required vaccination frequency, considering age and requirements of the National Vaccination Programme of all 225 0-14 year old Rotterdam Cape Verdians who were not vaccinated sufficiently against polio (less then three times). The 9 children who were on Cape Verde during the polio-epidemic are indicated between brackets. The number of non protected children is calculated according to the known efficacy of polio vaccination.[14]

Required vaccination frequency	Observed vaccination frequency				
	0	1	2	≥ 3	Total
0	27	0	0	0	27
1	16	0	0	0	16
2	25	1	0	0	26
3	10 [1]	6	21	0	37
> 3	35 [2]	40 [4]	44 [2]	0	119
Total	113	47	65	0	225
Efficacy against poliomyelitis	0%	36%	89%	100%	
No protection against clinical poliomyelitis	113	30	7	0	150

No poliovirus was detected in the six sewage samples in the six neighbourhoods examined. In four of six samples other enteroviruses were detected.
In 2000 and 2001, no poliovirus infections were reported in The Netherlands. In an existing enterovirus surveillance programme OPV virus was found in faeces samples of four hospitalized patients of Cape Verdian origin, not admitted for symptoms of poliomyelitis.

Finally, no statistically significant difference was found between respondents and non-respondents aged 0–14 years and 0–30 years in vaccination frequency and proportionate spread throughout the Cape Verdian communities, respectively.

Discussion

Although no cases of polio infection were reported, risk factors for the introduction of poliovirus in the Cape Verdian community in Rotterdam were certainly present.

No poliovirus was detected in the faeces of 154 individuals in the combined study groups aged 0–30 years, indicating statistically that an infection rate of 1.1% at most was missed in this group. This rate is much lower than the infection rate of 6% found in The Netherlands during the early phase of the polio

epidemic in 1992–1993, when an average infection rate in faeces was found of 6% in schools with both vaccinated and non-vaccinated students.[15]

Also in the sewage investigation, no indication was found, for circulation of poliovirus among Cape Verdians living in Rotterdam.
Routine screening of sewage samples makes the prediction of a polio epidemic in its early stages possible.[12,15] The sewage investigations in Rotterdam were conducted in neighbourhoods with the highest concentration of Cape Verdians in Rotterdam (5700/14 449). With the sensitivity of the study by Hovi et al.[16] extrapolated to the Rotterdam sewage investigation, one poliovirus secretor among the 5700 Cape Verdians would have been detected.

Cape Verdians in Rotterdam, aged 15–30 years reported the high vaccination coverage of 90%. The coverage in the age groups 0–14 years was 94.6%, which is comparable with the coverage in the Dutch population in general. The remaining 5.4% was at least partly vaccinated.

Also, Cape Verdians in Rotterdam live well spread over the city and do not constitute >15% of any neighbourhood population. In particular, there was no concentration of insufficiently vaccinated Cape Verdians in Rotterdam.
Cape Verdians living illegally in Rotterdam were not included in the study, and thus could bias the results. According to key figures in the Cape Verdian community in Rotterdam however, illegal Cape Verdians add at most 10% to the Cape Verdian community and live concentrated in the six neighbourhoods from which the sewage samples were taken.
Besides their own herd immunity, Cape Verdians were protected as well by the high vaccination coverage of the general Dutch population in Rotterdam, which is approximately 97%.[1] The minimal required vaccination coverage to prevent circulation of poliovirus is 82–87%.[17]

Alertness regarding adequate vaccination of travellers to polio-epidemic areas remains necessary. The polio epidemic in Cape Verde was only reported to the health authorities in Rotterdam 3 months after it started. This underlines the importance of rapid communication of outbreaks to authorities.

This study showed that there was no need of fear for indirect transmission to the Orthodox Reformed religious communities in The Netherlands through the Cape Verdian community in Rotterdam.

Contacts between Cape Verdians and the Orthodox Reformed communities are limited anyway, because the latter attend their own schools and are unlikely to share hygienic facilities with Cape Verdians. Nevertheless, caution is warranted. In retrospect and considering the extensive coverage against polio in both the Cape Verdian and the general Dutch population, inactivated polio vaccine (IPV) vaccination of insufficiently vaccinated individual Cape Verdians would have been sufficient. In this way any conceivable risk of OPV virus to become pathogenic would be avoided.

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General discussion

chapter

9

This thesis focuses on outbreak management, the domain of public health practice, as an opportunity to obtain knowledge, basically the domain of science, for communicable disease control.

In the introduction we outlined the interrelationship between science and public health practice.

In the field of science, research leads to knowledge about determinants and interventions of communicable diseases on the levels of agent, transmission and host. In science, epidemiology is used to obtain evidence about determinants and interventions.

In public health practice knowledge is applied to control communicable diseases, and surveillance monitors the occurrence of communicable diseases and detects signals of outbreaks and combines signals to suspect an outbreak.

In outbreak management surveillance may lead to the formulation of hypotheses about determinants and possible interventions, and epidemiology is used for testing hypotheses for guidance towards effective control of communicable diseases at hand.

Answering the research questions of this thesis

The first research question is whether epidemiological studies during outbreak management are able to generate specific knowledge for the containment of the communicable disease at hand, and the second question is whether these studies are able to generate knowledge of more general nature than necessary for the immediate containment of the communicable disease at hand.

In short, the answer to these two questions is "yes". From my case studies, as described in the previous chapters of my thesis, this becomes evident. Systematic outbreak management, in which surveillance and epidemiology are combined, generates knowledge in the same way as research does in the domain of science.

To substantiate my confirmative answer to the two research questions, the case studies in my thesis and the knowledge obtained in these case studies are summarized in tables 9.1 and 9.2. The tables list the motivation for the studies and the knowledge that I have identified during the outbreak management.

Knowledge is differentiated into specific and general knowledge. Firstly each of the two types of knowledge are differentiated into the three basic determinants of communicable diseases: (1) the infectious agent and its source(s); (2) the transmission of the agent to the host, and the environment in which transmission takes place; and (3) the host, or a group of hosts, including the routes of entry into the host and the reactions (immunity, morbidity and mortality) of a host or a group of hosts to the infection with an agent. Secondly, the two types of knowledge are differentiated into interventions to control communicable diseases that are directed towards the three determinants of communicable diseases.[1-3]

Table 9.1 represents the pertussis studies in chapter 2-5. Chapter 2 describes an epidemiological study of an outbreak of coughing in a convent among the nuns and their staff in the village of Baexem, the Netherlands. The study started after the general practitioner of the convent reported to the surveillance system that for 9 weeks he had tried to diagnose an outbreak of coughing in the convent and to stop the outbreak with several therapies, including antibiotics. The general practitioner did not consider the diagnosis pertussis because the nuns were adults, while at that time pertussis was considered to be a children's disease.

The specific knowledge obtained in the course of the outbreak management was that pertussis was the cause of the outbreak. This was established in a retrospective cohort study. On the basis of this diagnosis, measures were taken to curb the outbreak.

The most important contribution to general knowledge for the control of pertussis was the discovery that adults can be seriously at risk for morbidity and mortality due to pertussis. The hypothesis that adults are a true risk group for pertussis was substantiated in this study. The pertussis outbreak was followed to its end and serological testing was done up to one year after the outbreak. This follow-up of the outbreak provided general knowledge about the practicality and yield of single and paired IgA and IgG antibody titers for diagnosing pertussis infections among adults.

The study described in chapter 2 served as a data base for three further studies of pertussis. The outcome of the serological testing of the cohort of nuns and their staff was used in the successive studies described in chapters 3 and 4 to obtain more general knowledge about the diagnosis of pertussis with serological tests. In these chapters we focused on the IgA and IgG antibody titer levels for

diagnosing pertussis infections and were able to contribute to general knowledge about the increase and decrease of IgG antibody titers in children and adults with clinical pertussis, and to general knowledge about the meaning of single IgA and IgG titers for laboratory confirmation of pertussis in adults in the first three weeks of pertussis.

In chapter 5 the outcome of the study in chapter 2 was linked to the outcome of a study of the careers of the nuns in the convent. This was done because in the study described in chapter 2 it was not found that within the group of elderly nuns the risk for pertussis was statistically significantly increased with age. It was therefore hypothesised that the serious outbreak among the elderly nuns could be seen as a natural experiment with lack of natural re-infections in the environment of the isolated convent. The linking of the data of the two studies helped to establish the association between social isolation and pertussis. The study described in chapter 5 revealed the importance of social isolation as a risk factor for pertussis. In that way general knowledge was obtained about environmental and host factors contributing to pertussis and leading to further insight into the importance of immunity due to natural re-infections. Finally, it also led to advice regarding possible vaccination strategies in countries with successful national vaccination campaigns.

Table 9.2 represents the outbreak studies in chapter 6-8.
The two studies on gastro-enteritis in chapter 6 and 7 revealed specific knowledge about the bacterial and viral cause of epidemics of gastro-enteritis. General knowledge was generated about ways to diagnose the cause of gastro-enteritis in the food and health care sector in order to avoid the risk of transmission and undesired prolongation of the duration of Norwalk-like virus outbreaks in this vulnerable sector. The discovery of *Salmonella typhimurium* in a salted and smoked meat product initiated further research into this possible risk factor by the Food and Consumer Product Safety Authority.
Finally, in chapter 8 the study on possible introduction of polio virus in Rotterdam through the resident Cape Verdian community generated knowledge about the risk of introduction of poliovirus into a migrant community in a major city during an outbreak of poliomyelitis in the country of origin. General knowledge was generated about the importance of travellers being adequately protected to avoid any risk of transmission of polio virus from epidemic and endemic countries towards countries with large unvaccinated populations. Knowledge was obtained as well about the use of inactivated polio vaccine (IPV) instead of live

Table 9.1. Summary of specific and general knowledge obtained from the pertussis study in Chapter 2, 3, 4 and 5.

Study		Chapter 2: An epidemic of pertussis among elderly people in a religious institution in The Netherlands
Motivation for the study		Outbreak management of a 9-week lasting epidemic of serious coughing in a convent with elderly nuns, not responding to antibiotic therapy given by the general practitioner
Specific knowledge		
Determinant level	Agent	*Bordetella pertussis* cause of outbreak in convent
	Transmission	Description of epidemiological curve of pertussis outbreak
	Host	Description of clinical signs and symptoms, frequency, and duration of the *Bordetella pertussis* outbreak among personnel and nuns in the convent Significant (4-fold) increasing and decreasing IgA and IgG titres used and accepted for confirmation of pertussis infection to overcome difficulties of confirmation due to the particular Dutch policy of pertussis testing Description of single IgA and IgG titre levels of subjects with and without clinical pertussis Description and comparison of yield of culture, PCR significant paired increasing and decreasing IgA and IgG tests Morbidity and mortality rates were significant lower among adult personnel than among elderly nuns
Intervention level	Agent	-
	Transmission	Antibiotics, as prescribed by the general practitioner of the convent, in the first 9 weeks of the epidemic, did not stop transmission of *Bordetella pertussis* in the convent
	Host	Antibiotics, as prescribed by the general practitioner of the convent, in the first 9 weeks of the epidemic, did not prevent serious morbidity and mortality due to *Bordetella pertussis* infection among nuns in the convent
General knowledge		
Determinant level	Agent	*Bordetella pertussis* causes pertussis among adults and elderly
	Transmission	-
	Host	
Intervention level	Agent	-
	Transmission	-
	Host	-

	Chapter 3: Age-specific long-term course of IgG antibodies to pertussis toxin after symptomatic infection with *Bordetella pertussis*	Chapter 4: Sensitivity and specificity of single IgA and IgG antibodies for early diagnosis of pertussis in adults: evaluation for outbreak management in public health practice	Chapter 5: A pertussis outbreak associated with social isolation among elderly nuns in a convent
	Interest in improvement of the serological pertussis test in children and adults, based on clinical pertussis among children and adults	Interest in improvement of the serological diagnostic test for early diagnosis of pertussis among adults in public health practice	Seeking of an explanation of the serious pertussis epidemic among elderly nuns and their personnel in a religious convent in 1992[4]
	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
	Comparison of increase and decrease of IgG antibody titres in children and adults with pertussis showed no difference in rise, peak and decline of IgG between different age groups	Low levels IgA and IgG titres are sensitive, specific and practical for laboratory confirmation of *Bordetella pertussis* in adults in the first three weeks of outbreak management	Age is not, and social isolation is a risk factor for pertussis
			Lack of natural re-infections with *Bordetella pertussis* suggested as cause of serious pertussis epidemic
	-	-	-
	-	-	-
	-	-	Suggestion made that booster doses of pertussis vaccine may be a valuable strategy to control pertussis in populations with successful national childhood vaccination campaigns

Table 9.2. Summary of specific and general knowledge obtained from the outbreak studies in Chapter 6, 7 and 8.

Study		Chapter 6: An epidemic of *Salmonella typhimurium* associated with traditional salted, smoked, and dried ham
Motivation for the study		Outbreak management of an epidemic of gastro-enteritis among guests of a family party
Specific knowledge		
Determinant level	Agent	*Salmonella typhimurium* fage type 20 cause of the outbreak of gastro-enteritis
	Transmission	Salted and smoked ham was source and vehicle of transmission of *Salmonella typhimurium*
	Host	Description of clinical signs and symptoms, frequency, incubation period and duration of a *Salmonella typhimurium* infection
Intervention level	Agent	
	Transmission	
	Host	
General knowledge		
Determinant level	Agent	
	Transmission	
	Host	
Intervention level	Agent	Salting and smoking appeared insufficient bactericidal to kill *Salmonella typhimurium* in pig meat
	Transmission	
	Host	

Chapter 7: A gastroenteritis epidemic caused by a Norwalk-like virus after two weddings in a restaurant; a plea for integral microbiological investigation	Chapter 8: Risk of introduction of poliovirus into a Dutch Cape Verdian community during an outbreak of poliovirus in Cape Verde, 2000
Outbreak management of an epidemic of gastro-enteritis among guests after a wedding party	To assess the risk of introduction of polio virus in a Cape Verdian community in Rotterdam, during the polio epidemic in Cape Verde in 2000, before live oral polio vaccine (OPV) was used in a mass vaccination campaign in the Cape Verdian community in Rotterdam. The mass vaccination campaign was held to prevent poliomyelitis in individual cases, and to prevent transmission of the poliovirus to the non-vaccinated communities in the Bible Belt in the Netherlands
Norwalk-like virus cause of outbreak of gastro-enteritis	No evidence of polio virus infection was found in the Rotterdam Cape Verdian community
Description of epidemiological curve of Norwalk-like virus outbreak	Unvaccinated subjects travelled between the Cape Verdian community in Rotterdam and the polio-epidemic Cape Verde, risking transmission of poliovirus between the two countries
Important guest in the wedding party appeared to be the index case of the Norwalk-like virus outbreak	
Description of clinical signs and symptoms, frequency, incubation period and duration of a Norwalk-like virus infection	
	Distribution of sufficiently and insufficiently vaccinated Cape Verdians in the general population of Rotterdam is proportionately sufficient to maintain herd immunity against poliomyelitis No evidence was found to justify the use of live oral polio vaccine (OPV) in a an anonymous mass vaccination campaign in a Rotterdam Cape Verdian community with HIV-infection rates up to 5% in some risk groups
During gatherings like parties Norwalk-like virus is being transmitted directly from person to person	
Testing of food products, feces and the environment for bacterial and viral causes might increase the speed of diagnosing Norwalk-like virus as cause of outbreaks of gastro-enteritis in the food industry and the health care. This will accelerate the execution of hygienic measures to stop the spread of Norwalk-like virus	
	Alertness regarding adequate vaccination of travellers to polio-epidemic areas remains necessary as long as poliomyelitis is not eradicated, especially of travellers between polio-endemic and polio-epidemic areas and the large insufficiently vaccinated population in the Netherlands
	In comparable situations a mass vaccination campaign with inactivated polio vaccine (IPV) has to be considered seriously in insufficiently vaccinated subjects in communities with a high IPV-vaccination coverage to avoid any conceivable risk of OPV virus to become pathogenic

oral polio vaccine (OPV) in comparable situations to avoid any conceivable risk of OPV virus to become pathogenic and to circulate as was detected in several countries since 1982.[4-8]

Limitations and strengths of outbreak studies reported in this thesis as sources of knowledge

Limitations

The most important limitation of outbreaks as sources for knowledge in general, and therefore also for the studies described in this thesis, lays partly in the reaction of the public, politicians and public health authorities responsible for public health, and partly in the urgency and the local situation in which an outbreak takes place.[2,3,9] These reactions may not match the circumstances that epidemiological studies of outbreaks and research require. If outbreaks cannot be investigated in the required circumstances, the outcome of epidemiological studies may have limited value. Therefore commitment of the responsible politicians and the public health authorities is required, as well as of the medical institutions and laboratories involved. Also the cooperation and commitment of the public is necessary, especially the population at risk in an outbreak study. Commitment is also needed by the organisation that is being investigated as a possible source of an outbreak.

The urgency of the situation of an outbreak not only requires a swift reaction from all persons responsible for the outbreak study, but also professionalism. Possible research methods which can be used in these urgent circumstances should be known. Also formulation of hypotheses and knowledge about unresolved scientific problems that can be investigated during an outbreak requires professionalism.

There is also a need for medical knowledge of clinical signs and symptoms of communicable diseases. This need for professionalism is clearly recognised for situations in which bioterrorism is at hand. In that case syndromic surveillance systems are essential as "smoke detectors", which require prompt outbreak investigation. For such circumstances the importance of the availability of well-trained public health professionals with responsibilities beyond the resistance of bioterrorism is emphasised and generally accepted.[10] However, the impact of non-bioterroristic communicable disease outbreaks on the health of populations

is much larger, and therefore justifies the highest standards of professional quality and attention towards outbreak management.

Strengths

In the introduction of this thesis I have argued that outbreaks should be considered as natural experiments of communicable diseases, which may reveal clues about the control of such diseases.

The major strength of my outbreak studies was the epidemiological approach. In this approach the steps for seeking a common denominator and defining a population at risk are important. Equally important is the formulation of a hypothesis about the nature of the agent causing the outbreak, its source, transmission route and port of entry, possible ways of control, and about the susceptibility of the host, or the group of hosts. Based on these steps, hypotheses can be tested by determining infection and illness rates in persons exposed and not exposed to potential source(s), by applying questionnaires, interviews and laboratory tests. In that way knowledge can be generated of more general nature than strictly necessary for the containment of the specific communicable disease at hand.

Surveillance is regarded to be a cornerstone of the system to control communicable diseases in a population, especially through tracking disease trends, identifying new disease threats, spotting serious outbreaks and monitoring control measures. But even in a country like England that is well known for its communicable disease control, the surveillance system does not meet these expectations.[11] Consequently, surveillance leads to uncertainty about the validity of reported numerators and about the correctness of chosen denominators to obtain valid and meaningful rates, as derived from reported diseases. This uncertainty about surveillance figures makes surveillance more suitable for hypotheses formulation than for hypothesis testing.[12]

Through investigating outbreaks with epidemiological studies, hypotheses formulated through data derived from surveillance may be tested. In that way epidemiological studies of outbreaks may compensate for the limitations of surveillance.

In my epidemiological studies of outbreaks hypotheses were tested about the cause of the outbreaks,[13-18] but also about risk factors like age,[16,17,19] type of

vaccine,[17] isolation from society,[18] ways of preparing food,[13,14] and environmental risk factors,[20] and about diagnostic methods.[21,22]

In the literature more examples are found of epidemiologic studies of outbreaks which generated specific and general of knowledge of determinants and interventions of communicable diseases important for the control of communicable. A recent example is a study of an observed increase in the reported incidence of *Salmonella typhimurium* by the Dutch surveillance system, which was followed by an epidemiological outbreak investigation.[23] In this particular study the cause of the outbreak was found and an intervention was proposed based on a hypothesis that could be tested with the epidemiological study. Another example is a study in which the hypothesis could be rejected that the highly pathogenic avian influenza A virus subtype H7N7 was not infectious for humans. An unexpectedly high number of transmissions of avian influenza A virus subtype H7N7 to people who were directly involved in handling infected poultry was observed, and evidence was found for person-to-person transmission.[24] The study was done during the outbreak of the H7N7 avian influenza in the Netherlands in 2003.

Conclusions and recommendations

1. Outbreaks should be considered as natural experiments of communicable diseases, which may reveal clues for the control of such diseases. Every outbreak should be considered for epidemiological investigation to test hypotheses, including any hypothesis that has been formulated as result of surveillance data. Any specific or general knowledge resulting from such outbreak management studies may be deployed in future primary and secondary prevention activities, including early diagnosis, detection, and containment of the communicable disease.

   It is therefore recommended to follow the steps given in Table 1 of this thesis for decision making about the epidemiological study of an outbreak.

2. Data gathered in an epidemiological outbreak study can serve as a data base for further investigation of the communicable diseases at hand. It creates the opportunity to combine these data with other data obtained about the communicable disease at hand, in order to obtain more general knowledge.

   It is therefore recommended to consider every outbreak as a potential data base for further investigation of the communicable disease.

3. Communicable disease control and particularly outbreak management deserves highly-trained medical and epidemiological professionals. These professionals deserve an environment in which public health practice and science can be integrated for the benefit of communicable disease control.

   Therefore an academic workplace, which combines the medical and academic surrounding of a university hospital and the surrounding of a public health service which is responsible for public health practice, is recommended as an ideal environment to combine the art and science of communicable disease control in populations.

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Summary

This thesis is about outbreaks of communicable diseases as natural experiments and as unique opportunities for obtaining knowledge, not only specific knowledge for the control of communicable diseases at hand, but also more general knowledge for future control of communicable diseases.

In **chapter 1**, the **general introduction**, we introduce communicable disease control as an important issue in public health.

We describe earlier experiences with epidemiological investigations of outbreaks of communicable diseases as source of inspiration for this thesis.
Subsequently we introduce the three basic determinants of communicable diseases (infectious agent, transmission and host) and explain how knowledge about these determinants will lead to the development of successful preventive interventions.

We present research of determinants of communicable diseases and the development of interventions as the domain of science, and the implementation of interventions (control) as the domain of public health practice.
Unsuccessful control may lead to outbreaks of communicable diseases, which are usually detected through surveillance systems. Outbreaks of communicable diseases are introduced as unique opportunities for research and as potential sources of knowledge. In this perspective we establish the need for an etiologic diagnosis to reach the goal of containment of an outbreak, argue that epidemiological evidence is necessary, and describe the systematic steps in outbreak management to be followed to reach this goal.

We introduce surveillance as a tool in the control of communicable diseases. The purpose of surveillance is to watch over the occurrence of communicable diseases. Surveillance is defined as the permanent process of systematic collection, orderly consolidation, analysis, interpretation and evaluation of pertinent data with prompt dissemination of the results and processed information to those who need to know, particularly those who are in a position to take action in cases of outbreaks to control a communicable diseases. We indicate the concern about the quality of data obtained in surveillance suffering from the "*garbage in, garbage out*" principle. Ways to improve surveillance are described. We state that surveillance data may be used to monitor health events and determinants, and

to formulate hypotheses, but not to test hypotheses. So surveillance should lead to, but not be confused with research.

We depict in a model the various aspects of communicable disease control and how the domains of science (research and the body of knowledge about determinants and interventions) and public health practice (surveillance, application of interventions and outbreak management) interrelate.

The general introduction ends with the research questions:

1. Are epidemiological studies during outbreak management able to generate specific knowledge for the containment of the communicable disease at hand?
2. Are epidemiological studies during outbreaks management able to generate knowledge of more general nature than necessary for the immediate containment of the communicable disease at hand?

In **chapter 2** we describe an outbreak of pertussis among elderly people in a religious institution in the Netherlands.

The study was started because an epidemic of serious coughing with a duration of 9 weeks, was not explained by the general practitioner of the institution. Antibiotics had not stopped the epidemic. In a first clinical evaluation of a group of coughing nuns the working diagnosis pertussis was made because the type of coughing was very similar to the typical pertussis coughing which can be observed in the tropics in unvaccinated children.

In the retro- and prospective epidemiological study of the outbreak, the subjects were evaluated for their vaccination status and for history and presence of respiratory symptoms. Specimens were collected from the 75 nuns and 24 staff members for the laboratory investigation cultures, polymerase chain reactions, and changes in antibody titers in reaction on an infection with agents likely to have caused the outbreak. Special attention was given to pertussis as cause of the epidemic because of the characteristic clinical symptoms of nuns in the convent.

None of the 75 residents and 19 of 24 non-resident personnel had been vaccinated against pertussis. The overall attack rate of clinical pertussis, defined as persistent cough lasting at least 2 weeks, was 49%. In five subjects with clinical

pertussis, either culture or polymerase chain reaction or both were positive for *Bordetella pertussis*. A significant (at least 4-fold) change in specific antibody titre was observed in 85% (41/48) and 20% (10/49) of subjects with and without clinical pertussis, respectively ($P<0.0001$, chi-square 41.1). The attack rate of laboratory-confirmed pertussis was 42% (41/98). This rate was 5% (1/19), 20% (1/5), and 53% (39/74) in vaccinated personnel, non-vaccinated personnel, and non-vaccinated residents, respectively (not significant). Among residents aged between 55–74 years and 75–94 years, the attack rates were 47% (17/36) and 58% (22/38), respectively (relative risk = 0.8; 95% confidence interval 0.5–1.3). Four of 75 residents (5%) died while they were symptomatic for pertussis.

It was concluded that the attack rate of pertussis was high among non-vaccinated elderly and that pertussis tended to increase with age and that there may be a considerable risk of mortality from pertussis in this population. Physicians were advised to be alert to the diagnosis of pertussis in the elderly with nocturnal and prolonged periods of coughing.

In **chapter 3** we describe a study in which we investigated whether and how the rate of decline of IgG antibodies to pertussis toxin (IgG-PT) after natural infection with *Bordetella pertussis* is dependent on age.

Therefore we measured IgG-PT in follow-up sera of 121 patients aged 0–94 years, obtained after 123 episodes of *Bordetella pertussis* infection.
For analysis we applied a dynamic model for the inactivation of *Bordetella pertussis* by the immune system.
There were no significant differences in rise, peak and decline of IgG-PT between different age groups, although there was a tendency for a more rapid increase, a higher peak and a faster decline with increasing age.

The IgG-PT cut-off of 100 U/ml for serodiagnosis of pertussis appeared valid in all age groups. A decline of IgG-PT to <10 U/ml was associated with increased risk of re-infection with *Bordetella pertussis*.

In **chapter 4** we state that an accurate and practical laboratory test is needed to confirm clinical diagnosis of pertussis in adults during the first 3 symptomatic weeks, when treatment is effective and transmission can be interrupted.

Therefore we focussed on the rise of IgA and IgG antibodies to pertussis in the earliest clinical stages of pertussis. We assessed the sensitivity and specificity of single IgA and IgG levels in the cohort study of a pertussis epidemic in the 99 adults in the closed religious community studied is chapter 2 and 3 as well. Sensitivities were assessed in the sera of 46 laboratory confirmed clinical pertussis cases during the first 3 weeks. Specificities were calculated in sera of 35 asymptomatic controls without clinical symptoms or laboratory confirmed infections from the same community (internal controls). We compared these specificities with the specificities of single IgA and IgG levels in 4275 external controls from a cross-section of the general Dutch population aged 21-79 years who had not coughed for more than 2 weeks in the past year, and without pertussis diagnoses. The study was done in the Netherlands when whole-cell pertussis vaccine was used in the national vaccination programme.

Levels of 24 U/ml for IgA and 27 U/ml for IgG gave sensitivities of 100% and 75%, respectively, in the first 2 weeks, 100% in the third week, and 97% after the fourth week. The levels were reached within 2 days after onset of increase, and remained above these levels for roughly 7.2 and 5.1 months, respectively. Specificity was 82% for IgA and 89% for IgG in the internal controls and 90% in the external controls, respectively.

We suggested levels of 24 U/ml for IgA and 27 U/ml (= 27 International Units (IU)/ml) for IgG as sensitive, specific, and practical for laboratory confirmation of clinical pertussis in adults in the first 3 weeks of outbreak management.

In **chapter 5** we describe a study attempting to reveal the explanation for the serious pertussis outbreak in the convent in the Netherlands in 1992, described in Chapter 2.
We showed that the pertussis incidence during the outbreak was higher among 75 retired (unvaccinated) nuns living in the convent (60%) than among 24 staff members (8%) living in the open society. We also showed that the pertussis incidence during the outbreak was higher among 9 nuns with only a convent career (100%) than among 66 nuns who had a career outside of the convent (55%) before retirement in the convent. The pertussis incidence increased with duration of social isolation but not with age. We concluded that natural infection with *Bordetella pertussis* contracted in society effectively protected nuns against pertussis. Indeed, in the Netherlands in 1995–1996, the estimated incidence of

infection was 6.6% per year among individuals 3–79 years of age, up to 10.8% among individuals 20–24 years of age, and much higher than the reported pertussis incidence of 0.01% in the Dutch population per year in the national surveillance system.

Because both infection-acquired immunity and vaccine-acquired immunity wane over time, we concluded that booster doses of pertussis vaccines may be a valuable strategy to control pertussis in populations with national childhood vaccination programs.

In **chapter 6** we describe an epidemic of gastroenteritis after a family party caused by *Salmonella typhimurium* and associate the epidemic with traditional salting, drying and smoking of raw pork meat.

To discover the cause of the epidemic of gastroenteritis we used a retrospective cohort study. All 109 party-goers were asked to complete a written questionnaire about consumed food products and demographic and clinical variables and to hand in a faeces sample. The data were collected at the Public Health Institute Midden-Limburg, the Netherlands. Faeces and the remaining food products were examined microbiologically. The attack rates and the incidence rates of positive faeces culture among consumers and non-consumers of specific food products were calculated as well as the corresponding relative risks (RR) with 95% confidence intervals (95% CI).

The overall attack rate among the participants in the study was 35% (30/86). Twenty-eight different food products were served. Only consumption of 'Coburger ham' and of 'bone ham' was statistically significantly related to being ill (RR: 2.4; 95% CI: 1.5-4.0 and RR: 1.4; 95% CI: 1.1-1.9, respectively). *Salmonella typhimurium* phage type 20 was statistically significantly more frequently found in faeces of ill compared with non-ill party-goers (RR: 6.4; 95% CI: 2.5-16.1). *Salmonella typhimurium* phage type 20 was found in 'Coburger ham'. Consumption of 'Coburger ham' only, was statistically significantly related to a positive faeces culture (RR: 4.1; 95% CI: 2.0-8.5). 'Coburger ham' and 'bone ham' originated from the same batch of raw meat and were prepared in the same manner in the same salt bath. The shorter duration of salting and drying of 'Coburger ham' compared with 'bone ham' corresponded with a higher relative risk of becoming ill.

We concluded that consumption of 'bone ham' and 'Coburger ham' infected with *Salmonella typhimurium* phage type 20 caused the outbreak. Traditional salting, drying and smoking of raw pork meat was not antimicrobiologically effective against *Salmonella typhimurium*.

We recommended investigation of the antimicrobiological effect of the traditional preparation of meat and underlined the importance of Good Manufacturing Practices and quality control in all stages of production of pork meat, according to the principles of Hazard Analysis and Critical Control Points.

Chapter 7 describes a gastroenteritis epidemic caused by a Norwalk-like virus after two weddings in a restaurant and provides a plea for integral microbiological investigation.

To elucidate the cause of the epidemic of gastroenteritis amongst the guests and waiting staff of two weddings celebrated on the same day in the same restaurant, we used a retrospective and descriptive epidemiological study. After an outbreak of gastroenteritis amongst 215 wedding guests and restaurant staff in 1999, we inventoried in the Public Health Service Midden-Limburg, the Netherlands, the demographic and clinical variables and consumed foods by means of a structured written interview. Faecal samples and remaining food products were bacteriologically examined, and later on faeces were virologically examined for the presence of the Norwalk-like virus (NLV). The attack and the incidence rates of NLV-positive faecal specimens amongst consumers and non-consumers of specific food products were calculated, as well as the corresponding relative risks with 95% confidence intervals.

The overall attack rate was among the weddings guests and personnel who participated in the study was 66% (102/155). Of the 61 different dishes served, two showed a statistically significant relation with being ill. These food products were eaten by 26% of the ill persons. An NLV with the same genotype was found statistically significantly more frequently in faecal specimens of ill persons compared with non-ill individuals. No statistically significant association was found between NLV-positive faeces and consumption of a specific dish. The index case began having symptoms of gastroenteritis at the morning of the wedding party, before food was served. The guests of the two wedding parties used the same entrance and toilets.

We concluded that the epidemic was caused by a single NLV strain. No association could be found between the consumption of certain food products and being ill. The NLV epidemic was probably caused through direct or indirect contact between wedding guests and restaurant personnel.

We recommended that in the case of such reports of gastroenteritis epidemics, to test faecal, food product and environment samples for both bacteria and viruses, with an emphasis on NLV, to ensure early diagnosis.

In **chapter 8** we describe the risk of introduction of poliovirus into the largest Cape Verdian community in the Netherlands during an outbreak of poliovirus in Cape Verde in 2000.

The study was carried out in the week before all against poliomyelitis insufficiently vaccinated 0-30 year old Cape Verdians in the Netherlands, were called upon, possibly anonymous, to be vaccinated with life oral polio vaccine (OPV). In the Dutch national vaccination program inactivated polio vaccine (IPV) is being used.

The purpose of the campaign was to protect Cape Verdians in The Netherlands against poliomyelitis and to combat transmission of polio virus to the Orthodox Reformed communities in the Netherlands.

We surveyed all 225 insufficiently vaccinated 0–14-year-old among all Cape Verdian children in Rotterdam (n = 4188) and a random sample of 285 out of all 15–30-year-old Cape Verdians (n = 5074) in Rotterdam, to assess travel behaviour and vaccination coverage. Faecal specimens were collected and sewage samples taken in neighbourhoods with a sizable Cape Verdian population for testing of polio virus.

Of the Cape Verdian children aged 0–14 years living in Rotterdam, 94.6% was sufficiently vaccinated, but 10% of the 91 responding insufficiently vaccinated children aged 0-14 years, reported travelling to Cape Verde during the polio epidemic. Of the responding adults aged 15–30 years living in Rotterdam (n=82), 10% was not vaccinated against polio, and 17% reported travelling to Cape Verde during the polio epidemic.

In the faeces of 80 insufficiently vaccinated individuals aged 0–14 years and in 74 adults aged 15–30 years, no poliovirus was detected. Samples of sewage from six sites were negative for poliovirus.

We concluded that, although risk factors for the introduction of polio virus were present, there was no evidence of poliovirus infection found in the Cape Verdian population in Rotterdam despite extensive travel to the Cape Verde during the outbreak.

We stated that, in retrospect and considering the extensive coverage against polio in both the Cape Verdian and the general Dutch population, inactivated polio vaccine (IPV) vaccination of insufficiently vaccinated individual Cape Verdians would have been sufficient. In this way any conceivable risk of OPV virus to become pathogenic would be avoided.

We underlined that alertness regarding adequate vaccination of travellers to polio-epidemic areas remains necessary and that in this perspective caution is warranted, especially regarding the ten of thousands members of the Orthodox Reformed communities in the Netherlands, not being protected against polio, as long as poliomyelitis is not eradicated completely from the world.

Finally, in **chapter 9**, the **general discussion**, the research questions are answered and the limitations and strengths are discussed in the context of literature.

We state that through investigating outbreaks with epidemiological studies, hypotheses, including hypotheses formulated through data derived from surveillance, may be tested. In that way epidemiological studies of outbreaks may compensate for the limitations of surveillance.

The conclusions and recommendations derived are:

1. Outbreaks should be considered as natural experiments of communicable diseases, which may reveal clues for the control of such diseases. Every outbreak should be considered for epidemiological investigation to test hypotheses, including any hypothesis that has been formulated as result of surveillance data. Any specific or general knowledge resulting from such outbreak management studies may be deployed in future primary and secondary prevention

activities, including early diagnosis, detection, and containment of the communicable disease.
It is therefore recommended to follow a set of steps given in the first table of this thesis for decision making about the epidemiological study of an outbreak.

2. Data gathered in an epidemiological outbreak study may serve as a data base for further investigation of the communicable diseases at hand. It creates the opportunity to combine these data with other data obtained about the communicable disease at hand, in order to obtain more general knowledge.

   It is therefore recommended to consider every outbreak as a potential data base for further investigation of the communicable disease.

3. Communicable disease control and particularly outbreak management deserves highly-trained medical and epidemiological professionals. These professionals deserve an environment in which public health practice and science can be integrated for the benefit of communicable disease control.

   Therefore an academic workplace, which combines the medical and academic surrounding of a university hospital and the surrounding of a public health service which is responsible for public health practice, is recommended as an ideal environment to combine the art and science of communicable disease control in populations.

Samenvatting

In dit proefschrift worden uitbraken van overdraagbare ziekten beschouwd als natuurlijke experimenten en als unieke kansen om kennis te verkrijgen, niet alleen kennis voor de directe bestrijding van overdraagbare ziekten, maar ook kennis van meer algemene aard voor toekomstige bestrijding van overdraagbare ziekten.

In hoofdstuk 1, de **algemene inleiding**, wordt de bestrijding van overdraagbare ziekten gepresenteerd als een belangrijk onderdeel van de maatschappelijke gezondheidszorg.

Eerdere ervaringen met epidemiologisch onderzoek van overdraagbare ziekten worden beschreven als bronnen van inspiratie voor dit proefschrift.

Daarna worden de drie basale determinanten (bepalende factoren) van overdraagbare ziekten gepresenteerd: het agens, de transmissie en de gastheer. We leggen uit hoe kennis over deze determinanten kan leiden tot de ontwikkeling van succesvolle preventieve interventies.

We presenteren onderzoek naar determinanten en interventies van overdraagbare ziekten als het domein van de wetenschap en het toepassen van interventies als het domein van de maatschappelijke gezondheidszorg.

Niet succesvolle beheersing van overdraagbare ziekten kan leiden tot uitbraken, die meestal worden opgespoord in surveillancesystemen. Uitbraken worden gepresenteerd als unieke kansen voor onderzoek en als potentiële bronnen van kennis. Vanuit dit perspectief stellen we de noodzaak vast voor het vinden van een oorzaak om een uitbraak te kunnen beheersen en beredeneren we dat hiervoor epidemiologisch bewijs nodig is. We beschrijven de stappen die stelselmatig zouden moeten worden gevolgd om epidemiologisch bewijs te verkrijgen en uitbraken te beheersen.

Vervolgens presenteren we surveillance als een hulpmiddel bij het bewaken en beheersen van overdraagbare ziekten. Surveillance wordt gedefinieerd als "het voortdurend en stelselmatig verzamelen, rangschikken, ontleden, schatten en uitleggen van relevante cijfers, onmiddellijk gevolgd door verspreiding van de verkregen resultaten onder degenen die erover moeten weten, en in het bijzonder onder degenen die bij machte zijn om actie te ondernemen in geval van een uitbraak, om de overdraagbare ziekte te beheersen".

We wijzen dan op de bezorgdheid die bestaat over de kwaliteit van de cijfers verkregen in surveillance, waardoor surveillance lijdt aan het "*garbage in, garbage out*"-principe. Manieren om surveillance te verbeteren worden beschreven. We stellen dat gegevens verkregen uit surveillance wel gebruikt mogen worden om toezicht te houden op gebeurtenissen met betrekking tot gezondheid en ziekte en hun determinanten en voor de formulering van hypothesen, maar niet om hypothesen te toetsen. Dus surveillance kan wel leiden tot wetenschap, maar moet er niet mee worden verwisseld.

Vervolgens presenteren we een model met de verschillende aspecten van de beheersing van overdraagbare ziekten. In het model is weergegeven hoe wetenschap (onderzoek en het geheel aan kennis over determinanten en interventies) en daadwerkelijke maatschappelijke gezondheidszorg (surveillance, uitvoeren van interventies en het beheersen van uitbraken) samenhangen.

De algemene inleiding eindigt met de onderzoeksvragen:

1. Kan met behulp van epidemiologische studies bij het beheersen van een uitbraak specifieke kennis worden verkregen voor de directe beheersing van een overdraagbare ziekte?
2. Kan met behulp van epidemiologische studies bij het beheersen van een uitbraak, kennis worden verkregen, die van algemenere aard is dan nodig voor de directe beheersing van een overdraagbare ziekte?

In **hoofdstuk 2** beschrijven we een studie van een uitbraak van kinkhoest in een groep van ouderen in een klooster in Nederland.

De studie werd opgezet omdat de oorzaak van een al 9 weken durende epidemie van ernstig hoesten niet achterhaald was door de huisarts van het klooster. Antibiotica hadden de epidemie niet beëindigd.

Tijdens een eerste klinische evaluatie van een groep hoestende nonnen werd de werkdiagnose kinkhoest gesteld omdat het hoesten veel leek op het typische hoesten dat in de tropen kan worden waargenomen bij niet-gevaccineerde kinderen.

In een retro- en prospectieve studie van de uitbraak, werd de vaccinatiestatus en de aanwezigheid van en het verloop van hoesten onderzocht bij de 99 personen

in het klooster. Van deze 75 nonnen en 24 personeelsleden werden ook monsters verzameld voor laboratoriumonderzoek van kweken, polymerase-kettingreacties, en veranderingen in antistoftiters ten gevolge van besmetting met mogelijke oorzaken van de epidemie. Speciale aandacht werd gegeven aan kinkhoest als oorzaak, vanwege de karakteristieke klinische symptomen van de nonnen in het klooster.

Geen van de 75 nonnen en 19 van de 24 personeelsleden waren gevaccineerd tegen kinkhoest. Het percentage personen met klinische kinkhoest, gedefinieerd als een aanhoudende hoest van minstens 2 weken, was 49%. Van hen had 10% een positieve kweek, een positieve polymerase kettingreactie of beide. Bij 85% (41/48) van de personen met klinische kinkhoest en bij 20% (10/49) van de personen zonder klinische kinkhoest werd een significante (minstens 4-voudige stijging) van specifieke antistoftiters tegen kinkhoest waargenomen ($P<0.0001$, Chi-kwadraat 41.1). Het percentage personen met laboratorium bevestigde kinhoest bedroeg 42% (41/98). Dit percentage bedroeg respectievelijk 5% (1/19), 20% (1/5), en 53% (39/74) bij gevaccineerd personeel, niet-gevaccineerd personeel en de niet-gevaccineerde bewoners van het klooster (statistisch niet-significant). Dit percentage bedroeg bij de kloosterbewoners in de leeftijdscategorieën 55-74 jaar en 75-94 jaar respectievelijk 47% (17/36) en 58% (22/38), (relatieve risico = 0.8; 95% betrouwbaarheidsinterval 0.5–1.3). Vier van de 75 kloosterbewoners (5%) stierven toen zij leden aan de symptomen van kinkhoest.

We concludeerden dat het kinkhoestpercentage hoog was onder de niet-gevaccineerde oudere kloosterbewoners, dat kinkhoest neigde toe te nemen met de leeftijd en dat kinkhoest een aanzienlijk sterfterisico zou kunnen zijn in de populatie ouderen.
We adviseerden artsen om alert te zijn op het stellen van de diagnose kinkhoest bij ouderen met nachtelijk en langdurig hoesten.

In **hoofdstuk 3** beschrijven we een studie waarin we onderzochten of en hoe de antistof IgG pertussis toxine (IgG-PT) tegen kinkhoest afhankelijk van de leeftijd daalt, na een natuurlijke infectie met de *Bordetella pertussis* bacterie.

Daarvoor maten we het verloop van IgG-PT in de sera van 121 patiënten in de leeftijd van 0-94 jaar, die samen 123 episoden van een besmetting met *Bordetella*

pertussis doormaakten. Voor de analyse gebruikten we een dynamisch model voor de inactivatie van *Bordetella pertussis* door het immuunsysteem.

In de verschillende leeftijdgroepen werd er geen significant verschil gevonden in de stijging, piekhoogte en daling van IgG-PT, hoewel IgG-PT er toe neigde om met toename van de leeftijd sneller te stijgen naar hogere pieken en sneller te dalen.
Het IgG-PT afkappunt van 100 U/ml leek valide voor de sero-diagnose kinkhoest in alle leeftijdscategorieën.
Een daling van IgG-PT beneden 10 U/ml werd in verband gebracht met een toename van het risico op een re-infectie met *Bordetella pertussis*.

In **hoofdstuk 4** stellen we dat een nauwkeurige en praktische laboratoriumtest nodig is om bij volwassenen de klinische diagnose kinkhoest serologisch te kunnen bevestigen in de eerste drie symptomatische weken. Immers dan is behandeling effectief en dan kan transmissie van de *Bordetella pertussis* bacterie worden doorbroken.

Daarvoor spitsten we ons toe op de stijging en daling van IgA en IgG antistoffen in de vroegste klinische dagen van kinkhoest. Wij beoordeelden de sensitiviteit en specificiteit van enkelvoudige IgA en IgG niveaus in de cohortstudie van de kinkhoestepidemie onder de 99 volwassenen in de gesloten religieuze gemeenschap, die eveneens werd bestudeerd in hoofdstuk 2.

De sensitiviteit van IgA en IgG in de eerste 3 klinische weken van kinkhoest werd bepaald in de 46 personen met laboratorium bevestigde kinkhoest met klinische verschijnselen. De specificiteit van IgA en IgG werd berekend in de sera van de interne controle groep, de 35 personen zonder klinische symptomen van kinkhoest en zonder een laboratorium bevestigen infectie met *Bordetella pertussis*. We vergeleken deze specificiteiten met de specificiteiten van enkelvoudige IgA en IgG niveaus in de externe controlegroep, een dwarsdoorsnede van 4275 personen uit de algemene Nederlandse bevolking in dezelfde leeftijdscategorie van 21-79 jaar. Deze groep gaf aan, in het jaar voorafgaande aan het dwarsdoorsnedeonderzoek, niet langer dan 2 weken gehoest te hebben en geen diagnose kinkhoest te hebben gekregen. Het onderzoek werd uitgevoerd in Nederland toen het cellulaire vaccin tegen kinkhoest werd gebruikt.

Niveaus van 24 U/ml IgA en 27 U/ml IgG gaven een sensitiviteit van respectievelijk 100% en 75% in de eerste twee klinische weken van kinhoest, 100% in de derde week en 97% vanaf de vierde week. Deze IgA en IgG niveaus werden binnen 2 dagen na het begin van hun stijging bereikt, en ze bleven boven deze niveaus gedurende respectievelijk 7.2 en 5.1 maanden. De specificiteit van 24 U/ml IgA en 27 U/ml IgG was respectievelijk 82% en 89% in de interne controlegroep en 90% in de externe controlegroep.

We stelden een IgA niveau van 24 U/ml en een IgG niveau van 27 U/ml (= 27 Internationale Units (IU)/ml) voor als sensitieve, specifieke en praktische tests voor de laboratoriumbevestiging van klinische kinkhoest in de eerste 3 weken bij uitbraak management.

In **hoofdstuk 5** beschrijven we een onderzoek waarin wordt getracht een verklaring te vinden voor de ernstige uitbraak van kinkhoest in het klooster in Nederland in 1992, beschreven in hoofdstuk 2.

We toonden aan, dat de incidentie van kinkhoest hoger was bij de 75 gepensioneerde (ongevaccineerde) nonnen, die woonden in het klooster (60%), dan bij de 24 personeelsleden (8%), die woonden in de open samenleving. Ook toonden we aan, dat tijdens de uitbraak de incidentie van kinkhoest hoger was onder de 9 nonnen die hun hele carrière in het klooster verbleven (100%), dan onder de nonnen die een carrière buiten het klooster hadden, voordat ze met pensioen in het klooster terugkeerden (55%). De incidentie van kinkhoest nam statistisch significant toe met de duur van de sociale isolatie in het klooster en niet met de leeftijd van de nonnen.

Wij concludeerden dat natuurlijke infectie met *Bordetella pertussis* opgelopen in de samenleving de nonnen effectief beschermde tegen kinkhoest. Dit paste bij de geschatte incidentie van natuurlijke infecties met *Bordetella pertussis* in Nederland in 1995-1996 van 6.6% per jaar in de populatie in de leeftijdscategorie 3-79 jaar. Deze incidentie liep zelfs op tot 10.8% onder 20-24 jarigen. Deze infectie-incidentie was veel hoger dan de incidentie van 0.01% van de jaarlijks aangegeven gevallen van kinkhoest uit de algemene Nederlandse bevolking.

Omdat zowel immuniteit na infectie als na vaccinatie afneemt in de tijd, concludeerden we, dat het boosteren met pertussis vaccinatie mogelijk een waardevolle

strategie zou kunnen zijn in populaties met succesvolle vaccinatieprogramma's onder kinderen.

In **hoofdstuk 6** beschrijven we een onderzoek van een uitbraak van *Salmonella typhimurium* na een familiefeest. We brengen de uitbraak in verband met traditioneel gezouten, gerookte en gedroogde ham.

Om de oorzaak van de epidemie van gastro-enteritis te achterhalen voerden we een retrospectief cohortonderzoek uit vanuit de Gewestelijke Gezondheidsdienst Midden-Limburg. Daartoe inventariseerden we met een gestructureerde schriftelijke vragenlijst onder alle 109 feestgangers de demografische gegevens, klinische verschijnselen en genuttigde voedselsoorten. Feces en voedselrestanten werden microbiologisch onderzocht. Vervolgens werden de ziektefrequenties en de frequenties van positieve feceskweken onder gebruikers en niet-gebruikers van specifieke voedselsoorten en de relatieve risico's (RR's) met 95%-betrouwbaarheidsinterval (95%-BI) berekend.

Het ziektepercentage in de groep die deelnam aan het onderzoek bedroeg 35% (30/86). Er werden 28 voedselsoorten geserveerd. Alleen het nuttigen van Coburger ham en van beenham was statistisch significant gerelateerd aan ziek worden (RR: 2,4; 95%-BI: 1,5-4,0 respectievelijk RR: 1,4; 95%-BI: 1,1-1,9). *Salmonella typhimurium* faagtype 20 werd statistisch significant vaker in feces van zieken dan van niet-zieken gevonden. (RR: 6,4; 95%-BI: 2,5-16,1). Alleen het nuttigen van Coburger ham vertoonde een statistisch significante relatie met het hebben van een positieve feceskweek (RR: 4,1; 95%-BI: 2,0-8,5). *Salmonella typhimurium* faagtype 20 werd in de Coburger ham aangetroffen. Coburger ham en beenham werden op dezelfde wijze bereid van dezelfde partij rauw varkensvlees in hetzelfde pekelbad. De kortere duur dat Coburger ham werd gepekeld en gedroogd vergeleken met beenham paste bij het hogere relatieve risico op ziek worden.

We concludeerden dat de oorzaak van de uitbraak het consumeren van met *Salmonella typhimurium* faagtype 20 besmette beenham en Coburger ham was. Het ambachtelijk zouten, drogen en roken had onvoldoende antimicrobiologisch effect tegen reeds in het rauwe varkensvlees voorkomende *Salmonella typhimurium*.

We adviseerden om nader onderzoek te doen naar het antimicrobiologische effect van het ambachtelijke procédé en onderstreepten het belang van de kwaliteits-

controle volgens de principes van 'hazard analysis and critical control points' in alle stappen van de productie van varkensvlees.

In **hoofdstuk 7** beschrijven we een uitbraak van maag- en darmstoornissen, veroorzaakt door een Norwalk Like virus (NLV) na twee bruiloften in een restaurant, en houden we een pleidooi voor integraal microbiologisch onderzoek.

Om de oorzaak te achterhalen werd vanuit de Gewestelijke Gezondheidsdienst Midden-Limburg een retrospectief en beschrijvend onderzoek uitgevoerd onder de 215 bruiloftsgasten en het restaurantpersoneel. Met een gestructureerde schriftelijke vragenlijst werden demografische gegevens, klinische verschijnselen en genuttigde voedselsoorten geïnventariseerd. Feces en voedselrestanten werden eerst bacteriologisch onderzocht; later werd de feces op de aanwezigheid van NLV onderzocht. De ziektepercentages en de frequenties van NLV-positieve uitslagen onder gebruikers en niet-gebruikers van specifieke voedselsoorten, en de RR met 95%-BI werden berekend.

Het ziektepercentage onder de bruiloftsgasten en het personeel die deelnamen aan het onderzoek bedroeg 66% (102/155). Er werden 61 verschillende gerechten geserveerd, waarvan 2 een statistisch significante relatie vertoonden met ziekte. Van deze 2 gerechten werd door 26% van de zieken gegeten. Een NLV met hetzelfde genotype werd statistisch significant vaker in feces van zieken dan in feces van niet-zieken aangetroffen. Er werd geen significant verband gevonden tussen het nuttigen van een specifiek gerecht en NLV-positieve feces.
De indexpersoon kreeg gastro-enteritis op de ochtend van de bruiloft vóórdat het voedsel werd geserveerd. De twee groepen bruiloftsgasten maakten gebruik van dezelfde ingang en toiletgroep.

We concludeerden dat de epidemie werd veroorzaakt door dezelfde NLV-stam. Omdat er geen verband werd gevonden tussen bepaalde typen voedsel en ziek worden, concludeerden we dat het virus waarschijnlijk verspreid werd door direct of indirect onderling contact tussen de bruiloftsgasten en het restaurantpersoneel.

We adviseerden om bij dit soort meldingen van epidemieën van gastro-enteritis fecesmonsters, voedselmonsters en omgevingsmonsters tegelijkertijd bacteriolo-

gisch en virologisch te onderzoeken, in het bijzonder op NLV, om de diagnose zo snel mogelijk te kunnen stellen.

In **hoofdstuk 8** beschrijven we het risico van import van het poliovirus in de grootste Kaapverdische gemeenschap in Nederland, tijdens de uitbraak van poliomyelitis (kinderverlamming) in Kaap Verdië in 2000. Het onderzoek werd uitgevoerd in de week voordat alle onvoldoende tegen poliomyelitis gevaccineerde 0-30 jarige Kaapverdiërs in Nederland werden opgeroepen om zich in een massavaccinatiecampagne, eventueel anoniem te laten vaccineren met het levende orale poliovaccin (OPV). In het Nederlandse Rijksvaccinatieprogramma wordt gevaccineerd met geïnactiveerd poliovaccin (IPV).

Het doel van deze campagne was om Kaapverdiërs in Nederland te beschermen tegen poliomyelitis en overdracht van het poliovirus naar de niet gevaccineerde Orthodox Gereformeerde gemeenschappen in Nederland tegen te gaan.

We onderzochten alle 225 onvoldoende gevaccineerde 0-14 jarige onder alle Kaapverdische kinderen (n=4188) in Rotterdam, en een willekeurige steekproef van 285 verkregen uit alle 15-30 jarige Kaapverdiërs (n=5074) in Rotterdam. We onderzochten het reisgedrag en de vaccinatiestatus en vroegen om een fecesmomster aan de onderzochte personen. In de buurten met een omvangrijke Kaapverdische bevolking werden rioolmonsters genomen.

Van de Kaapverdische 0-14 jarige kinderen in Rotterdam, was 94.6% voldoende gevaccineerd, echter 10% van de 91 responderende onvoldoende gevaccineerde 0-14 jarige kinderen rapporteerde een reis naar Kaap Verdië tijdens de polio-epidemie. Van de responderende 15-30 jarige Kaapverdische volwassenen in Rotterdam (n=82), rapporteerde 10% niet tegen polio te zijn gevaccineerd en 17% rapporteerde een reis naar Kaap Verdië tijdens de polio-epidemie.

In de feces van 80 onvoldoende gevaccineerde kinderen in de leeftijd van 0-14 jaar en van 74 volwassenen in de leeftijd van 15-30 jaar werd geen poliovirus gevonden. In de rioolmonsters van de 6 buurten werd geen poliovirus gevonden.

We concludeerden dat er wel risicofactoren aanwezig waren voor de import van poliovirus, maar dat er ondanks intensief reizen naar Kaap Verdië tijdens de

polio-epidemie, geen bewijs werd gevonden voor infectie van de Rotterdamse Kaapverdische gemeenschap met het poliovirus.
We stelden dat achteraf beschouwd en gezien de hoge vaccinatiegraad in zowel de Kaapverdische als de algemene Nederlandse bevolking met geïnactiveerd poliovaccin, het vaccineren van onvoldoende gevaccineerde Kaapverdiërs met geïnactiveerd poliovaccin, afdoende zou zijn geweest. Op die manier zou ieder denkbaar risico op het pathogeen worden van het levende OPV zijn vermeden.

We onderstreepten dat alertheid op afdoende vaccinatie van reizigers naar polio-endemische gebieden noodzakelijk blijft. We onderstreepten ook, dat in dit perspectief voorzichtigheid is geboden zolang tienduizenden leden van de Orthodox Gereformeerde gemeenschappen in Nederland niet beschermd zijn tegen kinderverlamming, en zolang kinderverlamming niet compleet is uitgeroeid op aarde.

Ten slotte worden in **hoofdstuk 9**, de **algemene discussie**, de onderzoeksvragen beantwoord en de sterke en zwakke kanten van het onderzoek besproken in de context van de literatuur.
We stellen, dat met epidemiologisch onderzoek van uitbraken hypothesen, inclusief hypothesen die zijn gebaseerd op data verkregen in surveillance, kunnen worden getoetst.
Op die manier kunnen epidemiologische studies van uitbraken compenseren voor de beperkingen van surveillance.

De conclusies en aanbevelingen zijn:

1. Uitbraken zouden moeten worden beschouwd als natuurlijke experimenten van overdraagbare ziekten, die aanwijzingen voor de beheersing van zulke ziekten kunnen onthullen.
   Bij iedere uitbraak zou daarom moeten worden overwogen of de uitbraak epidemiologische wordt onderzocht om wetenschappelijke hypothesen te toetsen, inclusief hypothesen die zijn gebaseerd op cijfers verkregen in surveillance. Specifieke of algemene kennis die voortkomt uit zulk onderzoek kan eventueel worden ingezet bij toekomstige primaire en secundaire preventieactiviteiten, met inbegrip van opsporing, vroege diagnostiek en de beheersing van de overdraagbare ziekte.

Daarom wordt aanbevolen om bij het nemen van een besluit over een epidemiologische studie van een uitbraak, de stappen te volgen die zijn weergegeven in de eerste tabel in dit proefschrift.

2. Gegevens die verzameld zijn in een epidemiologische studie van een uitbraak kunnen mogelijk dienen als gegevensbestand voor verder onderzoek van de overdraagbare ziekte. Zulk een gegevensbestand schept de mogelijkheid om het te combineren met andere gegevens verkregen over de overdraagbare ziekte, om zodoende meer algemene kennis te verkrijgen.

   Daarom wordt aanbevolen om iedere uitbraak te beschouwen als een potentieel gegevensbestand voor nader onderzoek van de overdraagbare ziekte.

3. De beheersing van overdraagbare ziekten en vooral het beheersen van uitbraken verdient bekwame medische en epidemiologische geschoolde vakmensen. Deze vakmensen verdienen een omgeving, waarin de praktijk van de maatschappelijke gezondheidszorg en de wetenschap kunnen worden verenigd, in het belang van de beheersing van overdraagbare ziekten.

   Daarom wordt een academische werkplaats, waarin zowel de medische als de academische omgeving van een universiteitsziekenhuis wordt gecombineerd met de omgeving van een dienst die verantwoordelijk is voor de uitoefening van de maatschappelijke gezondheidszorg, aanbevolen als een ideale omgeving om de kunst en de kunde van de beheersing van overdraagbare ziekten te combineren.

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Curriculum Vitae

Curriculum Vitae Paul Mertens

Paul Mertens is geboren in Bingelrade. Hij behaalt zijn HBS-b diploma aan het St.-Janscollege te Hoensbroek. Vanwege zijn belangstelling voor paleontologie en geologie gaat hij biologie studeren aan de Rijksuniversiteit van Utrecht, waar hij zijn propedeuse haalt.

Vervolgens studeert hij geneeskunde aan de Katholieke Universiteit van Nijmegen. Tijdens deze studie werkt hij in de verpleegkundige zorg in de geriatrische psychiatrie en de chirurgie. Zijn wetenschapsstage loopt hij in de sociale psychologie aan de Sorbonne in Parijs.

Na zijn artsexamen volgt hij de opleiding tot tropenarts aan het Koninklijk Instituut voor de Tropen te Amsterdam (Nationale Tropencursus voor Artsen en taalcursus Kiswahili), in het Bronovo Ziekenhuis te 's-Gravenhage (verloskunde en gynaecologie) en in het Sint Elisabeth Gasthuis in Arnhem (chirurgie).

Van 1980 tot 1984 wordt hij door het Ministerie van Buitenlandse Zaken uitgezonden naar het Keniaanse Ministerie van Volksgezondheid. Na werkzaam te zijn geweest op de afdelingen chirurgie en verloskunde en gynaecologie in het provinciale ziekenhuis van Nakuru wordt hij benoemd tot District Medical Officer van het Samburu District en later van het West Pokot District. Naast zijn klinische werk in het district ziekenhuis in Kapenguria werkt hij aan plannen voor de uitbreiding van de basisgezondheidszorg in West Pokot. Deze plannen worden ondersteund door het Nederlandse Ministerie van Ontwikkelingssamenwerking. Dit leidt in 1982 tot de oprichting van de Stichting Medische Hulp Kenia (SMHK), waarvan hij sinds 1987 voorzitter is. De SMHK draagt bij aan de uitbouw van de gezondheidszorg in West Pokot, ook via haar Keniaanse zusterorganisaties de Netherlands Harambee Foundations for Health and for Water.

In 1985 behaalt hij zijn Master in Public Health aan de Johns Hopkins University te Baltimore. Gedurende deze studie werkt hij in het National Institute of Health in Washington aan een onderzoek naar de relatie tussen prenataal foliumzuurgebruik en neurale buisdefecten. In het Radboud Ziekenhuis in Nijmegen werkt hij daarna als klinisch epidemioloog mee aan het opzetten van een studielijn over dit onderwerp.

Van 1986 tot 2000 werkt Paul Mertens bij de GGD Midden-Limburg te Roermond. In die periode volgt hij de opleidingen tot sociaal en forensisch geneeskundige (1988) bij de Stichting voor Sociale Gezondheidszorg te Utrecht en tot medisch leider rampterrein aan de Rijksbrandweeracademie (1992). In 1993 behaalt hij zijn Master of Science in de epidemiologie aan de Erasmus Universiteit te Rotterdam.
Bij de GGD Midden-Limburg vervult hij de functies van medisch coördinator van de afdeling Algemene Preventieve Gezondheidszorg en van plaatsvervangend directeur. Daarnaast werkt hij er als forensisch geneeskundige en medisch leider ambulancevervoer. Tevens verricht hij er onderzoek op het terrein van infectieziekten en antibioticaresistentie, medische milieukunde en preventieve tandheelkunde in samenwerking met de Universiteiten van Maastricht, Aken en Nijmegen. Vanuit een deeltijdaanstelling bij de Vakgroep Epidemiologie van de Universiteit Maastricht geeft hij onderwijs in de sociale geneeskunde.

In 1994 overlijdt zijn echtgenote Caroline Goderbauer. Hij neemt dan de zorg op zich voor zijn vier jonge zonen.

In 2000 gaat hij in de GGD Rotterdam e.o. werken als arts infectieziektenbestrijding. Vanuit deze functie wordt hij betrokken bij de opzet van grootschalige infectieziektenbestrijding in Nederland. Dit mondt uit in een deeltijdbenoeming tot senior stafmedewerker bij de Geneeskundige Hulpverlening bij Ongevallen en Rampen (GHOR) in de Veiligheidsregio Rotterdam Rijnmond. In deze veiligheidsregio werkt hij tevens in de rampenbestrijdingsorganisatie.

Dit proefschrift is geschreven in de academische werkplaats **Centre for Effective Public Health In the larger Rotterdam area (CEPHIR).** In deze academische werkplaats is Paul Mertens aangesteld als wetenschappelijk docent Public Health Practice bij de afdeling Maatschappelijke Gezondheidszorg van het Erasmus Medisch Centrum. Hij geeft er onderwijs in de infectieziektenbestrijding en de maatschappelijke gezondheidszorg en hij is praktijkopleider “arts maatschappij

en gezondheid". Hij is lid van werkgroepen en commissies van het Erasmus Medisch Centrum en van het Interfacultair Overleg Sociale Geneeskunde.

Beroepshalve is Paul Mertens bestuurslid van de Vereniging voor Infectieziekten Sectie Infectieziektenbestrijding en lid van de onderwijscommissie van de Koepel Artsen voor Maatschappij en Gezondheid, de Vereniging voor Epidemiologie en de Nederlandse Vereniging voor Tropische Geneeskunde en Internationale Gezondheidszorg.
Daarnaast is hij lid van de Raad van Advies van het bestuur van het Child in Need Institute, CINI-Holland.

Paul Mertens woont sinds 2000 met Marja van Strien en zijn kinderen in Gouda. Daar is hij lid van Kunst in de Kamer, de Historische Vereniging Die Goude en de Filosofische Kring Gouda.

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