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Virological NIVEL/RIVM surveillance of respiratory virus infections in the 1996/97 season

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156-176	Bureau Rapportenbeheer

CONTENTS

Mailing list 2

Abbreviations 6

Abstract 7

Samenvatting 8

1 Introduction
- 1.1 The impact of acute respiratory illnesses 9
- 1.2 Surveillance of ARI 10
- 1.3 The NIVEL/RIVM surveillance of ARI 11
- 1.4 The European Influenza Surveillance Scheme (EISS) 14
- 1.5 Reporting the data observed in the NIVEL/RIVM surveillance of ARI 14

2 Materials and Methods
- 2.1 Standard operating procedure (SOP) for taking nose/throat swabs from respiratory patients for the NIVEL/RIVM surveillance of ARI 16
- 2.2 Outline of the virological examinations 16
- 2.3 Outline of the bacteriological examinations 17

3 Results and Discussion
- 3.1 The course of the 1996/97 respiratory season in the Netherlands 18
- 3.2 Detections of viruses and bacteria: the numbers 26
- 3.3 Age dependency of virus and bacterium detection 33
- 3.4 Multiple infections 34
- 3.5 Prolonged shedding: examination of follow-up samples 36
- 3.6 Incidence of influenza-like illnesses (ILI) and influenza in the 1992/93 - 1996/97 seasons 37
  - 3.6.1 Incidence of influenza: the influenza correction factor 38
  - 3.6.2 Incidence of influenza: age dependency 39
  - 3.6.3 Incidence of influenza: dependency on influenza virus (sub)type and age 40
  - 3.6.4 Incidence of influenza: dependency on degree of urbanization 40
  - 3.6.5 Incidence of influenza: dependency on region 42
- 3.7 Age dependency of infections with all kinds of pathogens 43
- 3.8 Relation between clinical data and infection 45
  - 3.8.1 Association between clinical symptoms and specified infections 45
  - 3.8.2 Association between ILI and specified infections 49
  - 3.8.3 Association between clinical diagnoses and virus infections 49
  - 3.8.4 Association between specific patient conditions and virus infections 51

Conclusions 53

Acknowledgment 53

Literature 54

Tables and Figures
Table 1 Influenza viruses isolated in the Netherlands 1991-1997 19
Table 2 Summary of the results of virus/bacterium culture and -PCR of the NIVEL/RIVM GP surveillance of respiratory "viral" illnesses in the 1996/97 season (15 July 1996 - 13 July 1997) 28
Table 3 Results individual GPs 30
Table 4 Detection of respiratory viruses, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* in 540 nose/throat swabs from 540 patients in the 1996/97 season using virus isolation and PCR arranged according to week and pathogen (herpes simplex virus isolates not included) 31
Table 5 Bacteria by week 32
Table 6 Detection of viruses, Mp, and Cp in the 1996/97 season arranged according to age group 33
Table 7 Detection of conventional bacteria in the 1996/97 season arranged according to age group 34
Table 8 Multiple infections with virus/Mp/Cp in the 1994/95 and 1996/97 seasons 34
Table 9 Double and triple infections with combinations of virus/Mp/Cp in the 1994/95 and 1996/97 seasons 35
Table 10a Rhinovirus detection in clinical follow-up samples from RT-PCR-positive patients 37
Table 10b RS-virus detection in clinical follow-up samples from RT-PCR-positive patients 37
Table 11 Incidence of ILI per 10,000 inhabitants registered by NIVEL from week 40 up to week 20 arranged according to age group and season 39
Table 12 Calculation of laboratory-based correction factor for influenza incidences in the period 1992/93 up to and including 1996/97 arranged according to age group 39
Table 13 Estimated incidences of influenza per 10,000 inhabitants of the specified age group in the general population of the Netherlands arranged according to season 40
Table 14 Estimated incidences of influenza per 10,000 inhabitants of the specified age group in the general population of the Netherlands averaged over the seasons 1992/93 up to and including 1996/97 arranged according to virus (sub)type 41
Table 15 Incidence of ILI per 10,000 inhabitants registered by NIVEL calculated from week 40 up to week 20, arranged according to degree of urbanization and season 41
Table 16 Laboratory-proven influenza incidences in the 1992/93 up to and including 1996/97 seasons arranged according to urbanization 41

Table 17 Estimated incidences of influenza per 10,000 inhabitants in the general population of the Netherlands arranged according to degree of urbanization and season 42
Table 18 Incidence of ILI per 10,000 inhabitants registered by NIVEL, calculated from week 40 up to week 20, arranged according to province group and season 42
Table 19 Laboratory-proven influenza incidencesin the 1992/93 up to and including 1996/97 seasons arranged according to region 43
Table 20 Estimated incidences[1)] of influenza per 10,000 inhabitants of province group in the general population of the Netherlands arranged according to province group and season 43
Table 21 Detection of specified viruses in the period 1992/93 up to and including 1996/97 arranged according to age group 44
Table 22 Clinical symptoms of the virologically examined patients in the period 1992/93 up to and including 1996/97 arranged according to pathogen 46
Table 23 Clinical symptoms associated with influenza virus infections arranged according to (sub)type in the 1992/93 - 1996/97 period 47
Table 24 Odds ratios for clinical symptoms for the 1992/93 - 1996/97 period 48
Table 25 Virus profiles for ILI positive and ILI negative patients for the 1992/93 - 1996/97 period 50
Table 26 Virus/Mp/Cp detections arranged according to clinical diagnosis other than ILI (but often in combination with ILI) in the 1992/93 - 1996/97 period 50
Table 27 Association between detection of virus infections and allergy, contacts with 0 - 4 - year - old children, and similar infections in the neighbourhood 51

Figure 1 Compartments of infections 11
Figure 2 Geographic distribution of the sentinel GPs of NIVEL in the Netherlands 12
Figure 3 Respiratory 1996/97 season. Percentage of samples positive for any virus, rhinovirus, and influenza virus, respectively, per 4 weeks 20
Figure 4 Influenza-like illnesse (ILI) per 10,000 inhabitants per week among patients presenting to GPs in the Netherlands 1969 - 1996 (NIVEL) 21
Figure 5 Influenza epidemic 1996/97: ILI and virus isolates by (sub)type 22
Figure 6 Influenza epidemic 1996/97: ILI and virus isolates by origin 23
Figure 7 Influenza virus isolates from diagnostic labs versus GPs 1996/97 24
Figure 8 Influenza virus isolates from diagnostic labs versus GPs 1991 - 1997 25
Figure 9 Pie of results of NIVEL/RIVM surveillance of ARI 1996/97 29

Appendix 1 Poster presented at the symposium of the European Society for Clinical Virology in Hamburg on 30 August to 2 September 1998 58
Appendix 2 Questionnaire for NIVEL/RIVM surveillance of ARI 57
Appendix 3 Incidences ILI and ARI in Belgium 1995 - 1997 59

Abbreviations

ARI: acute respiratory virus infections
Cp: *Chlamydia pneumoniae*
EISS: European Influenza Surveillance Scheme
EUR: Erasmus University Rotterdam
GP: general practitioner
ILI: influenza-like illness
Mp: *Mycoplasma pneumoniae*
NIVEL: Nederlands Instituut voor Onderzoek van de Gezondheidszorg / Netherlands Institute of Primary Health Care
PCR: polymerase chain reaction
RIVM: Rijksinstituut voor Volksgezondheid en Milieu / National Institute of Public Health and the Environment
RS virus: respiratory syncytial virus
RT-PCR: reverse transcriptase step followed by polymerase chain reaction

Abstract

The purpose of the Netherlands Institute of Primary Health Care (NIVEL)/National Institute of Public Health and the Environment (RIVM) surveillance is to establish the incidence of acute respiratory virus infections (ARI) in patients who consult their family doctor because of ARI. Since the 1992/93 season, the general practitioners (GPs) of the NIVEL network have sent for this purpose nose-throat swabs from a selection of ARI patients to the RIVM. At the RIVM, these swabs were examined using virus culture and in the 1994/95 and 1996/97 seasons also using polymerase chain reactions (PCR) for the detection of selected viruses, *Mycoplasma pneumoniae* (Mp) and *Chlamydia pneumoniae* (Cp). In the 1996/97 season, part of the patients were also examined for conventional bacteria.

From week 29 of 1996 until week 28 of 1997, the RIVM received in total 540 clinical samples from 540 ARI patients from 36 of the 43 participating sentinel stations of the NIVEL. A potentially respiratory pathogenic agent was detected using culture and/or PCR in 64% of the specimens. Viruses were found in 55% and conventional bacteria in 16% of the samples (Table 2). Influenza virus, cultured from 24% of the samples, was the predominant virus, followed by rhinovirus (22%), respiratory syncytial (RS) virus (5%), and enterovirus (4%) (Table 2). Two respiratory pathogens were detected in 35 clinical samples and three respiratory pathogens in three samples (Table 8).

As in previous years, a temporary increase in the rate of positive samples - especially those containing rhinoviruses - was noted in September, coinciding with the opening of schools at the end of August (Fig. 3). The influenza epidemic of the 1996/97 season started in the second half of December in the Netherlands. It had the usual size (Fig. 4), and length (Fig. 5). The epidemic began with a major wave of subtype A(H3N2) virus infections, followed by a small overlapping wave of type B virus infections in weeks 4 - 9 (Fig. 5). Sixty-one percent of the influenza viruses isolated from GP patients were H3N2, while 88% of those isolated in diagnostic laboratories were this subtype (Fig. 7). In fact, this is a yearly recurring phenomenon (Fig. 8) and probably reflects the higher pathogenicity of subtype A(H3N2) compared with type B.

Over the five seasons studied, influenza virus infections accounted for at least 26% of the ILI registered by NIVEL (Table 25). Calculated over the same five seasons, an estimated 2.7% of the Dutch population developed an ILI caused by an influenza virus infection per season (Table 13). According to the ILI registration, the highest incidence of ILI was among 0-4-year-old children (Table 11). After correction for the influenza virus isolation rate and the fraction of ILI patients who consulted their GP, however, the highest incidence of influenza, 5.3%, was among the 5-14-year olds (Table 13). Influenza occurred most frequently in the (according to popular belief "healthy") country-side (T17) and least frequently in the northern region of the Netherlands (T18).

Samenvatting

Het doel van de surveillance van respiratoire virusinfecties van het Nederlands Instituut voor Onderzoek van de Gezondheidszorg (NIVEL) en het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) - is het vaststellen van de incidentie van acute respiratoire virusinfecties (ARI) bij patiënten die hun huisarts raadplegen wegens een ARI. Voor dit doel zenden huisartsen van het NIVEL-netwerk van huisartspeilstations sinds het seizoen 1992/93 van een selectie van hun ARI-patiënten neus-keeluitstrijken naar het RIVM. Op het RIVM worden deze onderzocht op de aanwezigheid van virussen door kweek en, in de seizoenen 1994/95 en 1996/97, ook door polymerase chain reaction (PCR) detectie-methoden op bepaalde virussen, *Mycoplasma pneumoniae* of *Chlamydia pneumoniae*. In 1996/97 werd een deel van de patiënten tevens onderzocht op conventionele bacteriën.

Tussen week 29 van 1996 en week 28 van 1997 ontving het RIVM van 36 van de 43 NIVEL-peilstations 540 klinische monsters afkomstig van 540 patiënten. In 64% van de monsters werd een virus (55%) of bacterie (16%) aangetoond (Tabel 2). Het vaakst werd influenzavirus (24%) aangetroffen, op de voet gevolgd door rhinovirus (22%). In 35 monsters waren twee pathogenen aanwezig, in drie monsters drie (Tabel 8).

Evenals in voorgaande jaren werd in september 1996 een verhoogd percentage positieve monsters waargenomen (Fig. 3). Deze verhoging viel samen met de aanvang van het nieuwe schooljaar. Zoals gewoonlijk begon in Nederland de influenzaepidemie half december 1996. Deze was normaal wat betreft omvang (Fig.4), en duur (Fig. 5). De epidemie begon met een golf van influenza subtype A(H3N2), in de weken 4 - 9 gevolgd door een kleine, overlappende golf van type B (Fig. 5). In week 8 werd één enkel A(H1N1) virus geïsoleerd. Bij de huisartspatiënten was 61% van de isolaten subtype A(H3N2), bij de isolaten van de virusdiagnostische laboratoria 88% (Fig. 7). Dit verschil is een jaarlijks terugkerend verschijnsel (Fig. 8) en waarschijnlijk het gevolg van de hogere pathogeniteit van subtype A(H3N2).

Berekend over de seizoenen 1992/93 tot en met 1996/97 werd een influenza-virus gekweekt bij tenminste 26% van de IAZ geregistreerd door het NIVEL (Tabel 25). Over dezelfde periode ontwikkelde per seizoen naar schatting 2.7% van de Nederlandse bevolking een IAZ veroorzaakt door dit virus (Tabel 13). Bij IAZ-positieve patiënten werden influenzavirussen driemaal vaker aangetoond dan bij IAZ-negatieve patiënten. Door de huisartsen van het NIVEL werd een IAZ het vaakst gezien bij kinderen van 0-4 jaar oud (Tabel 11). Na correctie voor het percentage monsters waaruit een influenza-virus werd gekweekt en voor de fractie IAZ-patiënten die de huisarts raadpleegt blijkt echter dat de influenzaincidentie het hoogst is bij 5-14 jarigen, nl 5,3% (Tabel 13). Influenza kwam het meeste voor op het - naar het volksgeloof zo "gezonde" - platteland (Tabel 17). De noordelijke regio werd het minst getroffen door deze ziekte (Tabel 18).

1. INTRODUCTION

1.1 The impact of acute respiratory illnesses

Acute respiratory illnesses (ARI) form a considerable part of the total disease burden of the general population. In a study in the USA, for example, the incidence of ARI was 2.5 per person per year (1). Upper airway complaints alone are the reason for about 25% of the primary visits to general practitioners (GPs) (2, 3). The large impact of ARI continuously urges the medical profession to investigate whether the control of these illnesses can be improved. Due to the limited sensitivity of diagnostic examinations, the fraction of ARI associated with the presence of infectious agents is not exactly known. It is, however, suspected to be high. For example, Monto and Sullivan estimated, using data from several reports, that viruses and bacteria are responsible for approximately 77% of ARI in the community (1). Using time-resolved fluoroimmunoassay, PCR, and serology, Mäkelä and colleagues demonstrated the presence of a virus (in the majority of the cases a rhinovirus) in 69% of the episodes of the common cold in a prospective study on young adults in Finland(4). In a longitudinal study, a British group found 80-85% of the exacerbations of asthma in 9-11-year-old children to be associated with a virus infection, again usually a rhinovirus infection (5).

As with other infectious diseases, control of infectious viral ARI is, in principle, possible by three approaches:

- Successful vaccination programs already exist for pertussis (whooping cough), diphtheria, and influenza. Vaccines against respiratory syncytial (RS) virus and parainfluenza virus are being developed.
- The antiviral drug amantadine is effective against influenza A virus infections. Adverse reactions, however, hamper more than sporadic use of this drug. Recently, less toxic agents, that inhibit influenza neuraminidase, were produced by Glaxo and Roche. These are promising new weapons in the fight against infections with influenza virus type A and B. The development of drugs against rhinoviruses lags behind those against the more serious influenza virus infections, but is still actively being pursued. Agouron, for instance, is developing an inhibitor of the enzyme 3C protease that is essential for rhinovirus multiplication.
- Changes in behaviour form a less promising route for control of viral ARI. Hygienic measures have little effect. Exclusion of ill pupils from schools or even the closure of schools is sometimes practised or considered in times of epidemics. The high percentage of subclinical infections and the fact that the virus is already widespread among the population when an epidemic is detected, however, render the success of these measures unlikely. On the other hand, for instance, the increasing use of private motorcars rather than public transportation delays the spread of respiratory agents without any specific interference by authorities.

The aetiology of infectious ARI is complex. Monto and Sullivan estimated that 70% of all ARI in the community are due to viruses and 8% to bacteria (1). In their estimation, rhinoviruses and coronaviruses alone cause 48% of all ARI. Other ARI-associated viruses include the three influenza viruses, the four parainfluenza viruses, RS virus, the 51 adenoviruses, and the 73 enteroviruses. Bacterial infections are estimated to cause 5-10% of all ARI (1, 6, 7). These include *Streptococcus pneumoniae* and *S. pyogenes*, *Haemophilus influenzae* and *H. parainfluenzae*, and *Moraxella catarrhalis* (7-11).

For effective control of ARI, it is essential to know the relative roles of the various agents. For instance, the larger the contribution of rhinoviruses to ARI, the more vigorously the pharmaceutical companies will pursue their efforts to develop suitable antiviral substances against these viruses.

1.2 Surveillance of ARI

Assessment of the total disease burden of ARI with regard to the individual viruses would mean determining the contribution of each virus to this burden at all levels of illness severity (Fig.1):

- Community level: illnesses that are not disturbing enough to lead to a visit to the physician.
- GP level
- Hospital level
- Fatal cases († in Fig. 1)

Causes of death are difficult to ascertain. Infections of hospitalized patients are already adequately covered by microbiological examinations.
Surveillance at the community level is rarely performed but is very expensive. We chose to examine the association between viruses and ARI at the GP level. The numbers are smaller than at the community level, but the severity is higher and, therefore, the total impact comparable. In the future, a community study should be considered to establish the differences in numbers, severity, and proportions of the various associated viruses compared with the GP level.

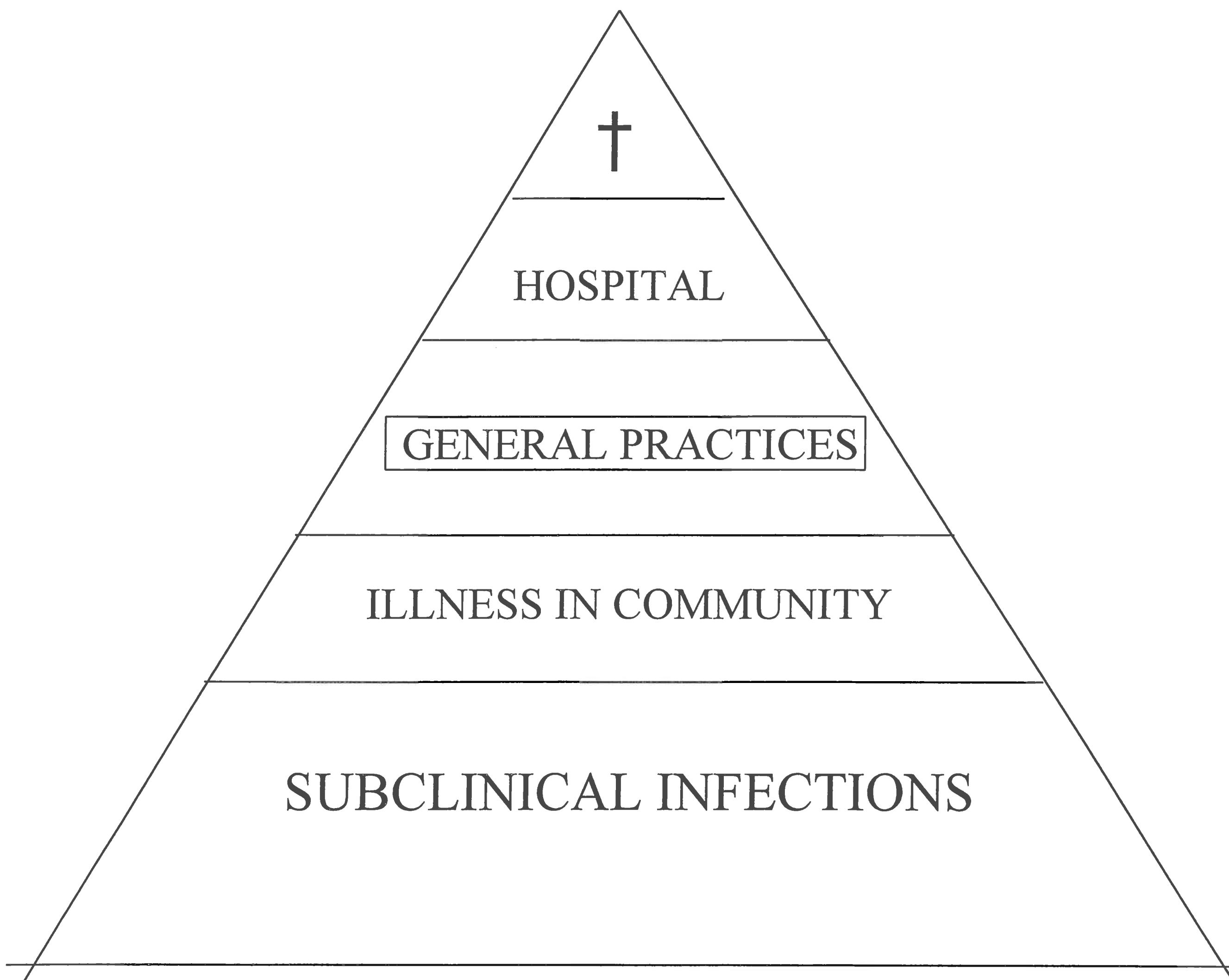


Figure 1. Compartments of infection

1.3 The NIVEL/RIVM surveillance of ARI

We used the Continuous Morbidity Registration of the NIVEL (12) for the ARI surveillance. Since 1970, the NIVEL has run a network of GPs, who record various events in their practices. In 1996, the network consisted of 43 sentinel stations staffed by 67 GPs. The distribution of the sentinel stations throughout the country is proportional to the regional population density (Fig. 2). They cover about 1% of the Dutch population, proportionally representing three classes of urbanization. During every respiratory season since 1970, the GPs have reported weekly the number of ILI cases to the central office of the NIVEL. Starting in 1992/93, the participating GPs were also asked to take, if possible, a nose and a throat swab from two respiratory patients and to forward them to the RIVM for virological examination.

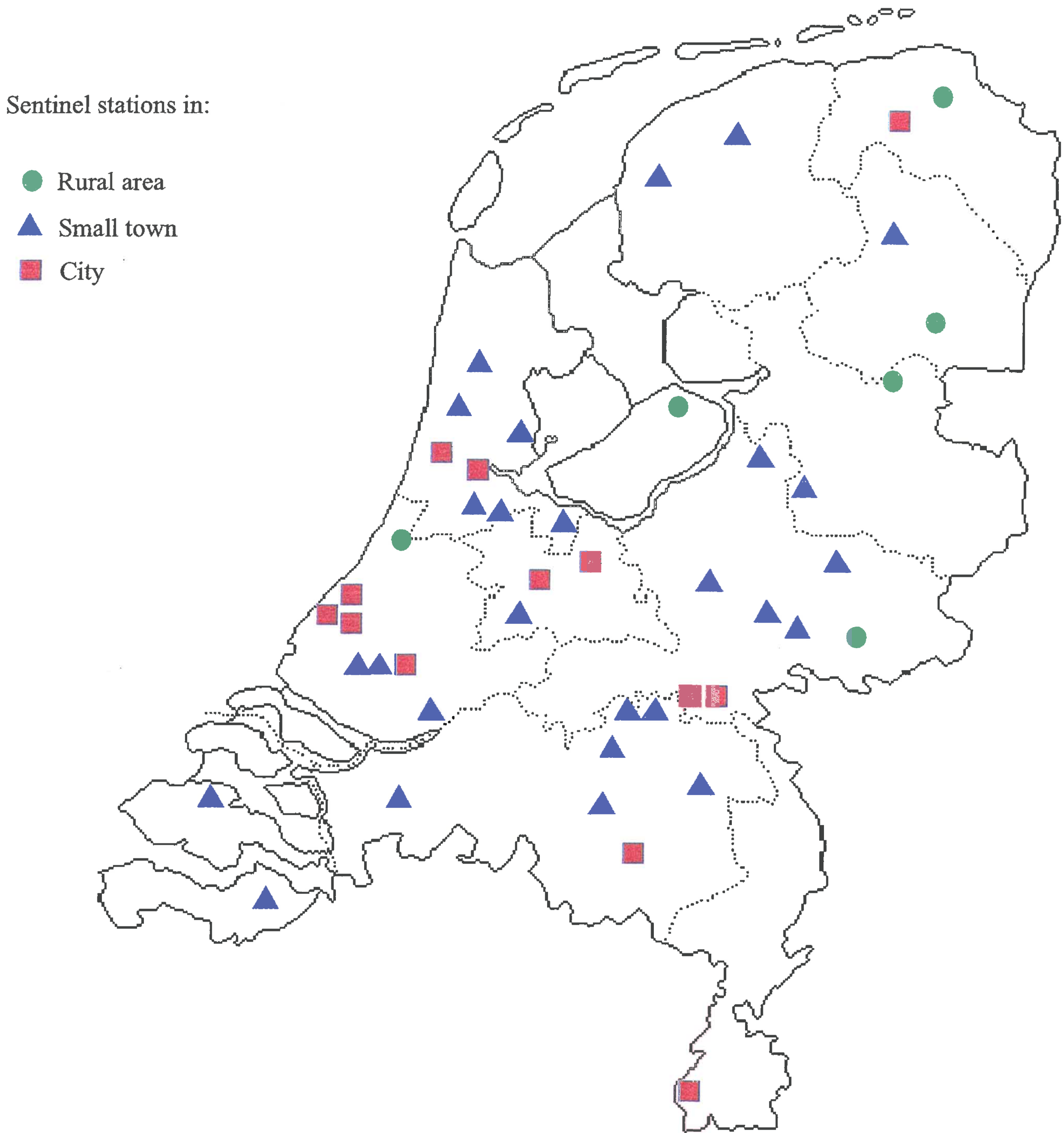


Figure 2. Geographic distribution of the sentinel Gps of NIVEL in the Netherlands

A major goal of the surveillance was to detect annually influenza epidemics at an early stage and to describe their aetiology. Influenza is defined as a disease with a certain spectrum of respiratory and general symptoms caused by one of the influenza viruses. Infection with one of the influenza viruses, however, does not necessarily lead to the syndrome. In about 25% of the cases, there is no significant illness (1, 13). Many other pathogens, however, can cause symptoms similar to those of the influenza viruses can do. In order to conduct a clinical surveillance of influenza among GP patients and to estimate the impact of influenza virus epidemics, the NIVEL adopted a series of criteria to discriminate between influenza virus infections and ARI from other causes and to make clear to GPs which patients should be registered as having an ILI. These criteria are (12, 14):

- Abrupt start of the disease: a prodromal phase with minor symptoms of less than five days
- Rectally measured body temperature of at least 38.0°C
- At least one of the following symptoms: cough, coryza, headache, retrosternal pain, myalgia

Because many influenza virus infections, especially those caused by (sub)types H1N1 and B, run a milder course, this definition leads to under-reporting of symptomatic influenza virus infections. Still, in practice, the NIVEL system yearly produces annual incidence curves for ILI that coincide well with periods of more-than-sporadic isolations of influenza viruses (15, 16, 17, and earlier RIVM reports). Due to the overlap with clinical symptoms induced by other infectious and even non-infectious factors, however, the incidence values of the NIVEL over-report influenza at the GP level. One of the main purposes of the present NIVEL/RIVM GP surveillance of respiratory viral illnesses, therefore, was to establish the fraction of ILI associated with influenza virus infection and estimate the real incidence of influenza in the Netherlands on the basis of both the registered incidence of ILI and a “laboratory-based correction factor”.

For the past twenty years, the ILI incidence has been low in the periods without influenza epidemics, namely about 2-4 cases per 10,000 of inhabitants, and much higher during the epidemics, 20 - 70 cases per 10,000 of inhabitants. In particular, in the absence of influenza, the ILI incidence registered by the NIVEL does not respond to the annual epidemic of RS virus round the turn of the year. Waves of increased ARI do, however, occur during these interepidemic periods and public health authorities, physicians, and the general public are interested in knowing the culprits of these inconveniences. Since these cases of ARI rarely lead to hospitalization, the usual hospital-based virus diagnostic activities fail to answer this question. The of ARI can provide information in this area.

Another goal of the NIVEL/RIVM surveillance is the characterisation of influenza virus strains from patients consulting their GP. These strains may differ from those obtained in the diagnostic laboratories, which mainly deal with specimens from hospitalized patients. In fact, they do (18). Therefore, the antigenic reactivity of the influenza virus strains isolated in the NIVEL/RIVM surveillance were analysed in detail at RIVM in close collaboration with the Erasmus University Rotterdam (EUR) in the framework of the NIC (National Influenza Centre of the Netherlands, which is a collaboration of the RIVM and the EUR.

1.4 The European Influenza Surveillance Scheme (EISS)

The need for early detection and characterization of the annual influenza epidemics has also prompted health authorities abroad to establish surveillance systems comparable to the NIVEL/RIVM surveillance of ARI. A few years ago, the national networks of Belgium, England, France, Germany, Portugal, and Spain started to exchange and integrate their data in a collaborative activity called European Influenza Surveillance Scheme (EISS). Recently, Switzerland, the Czech Republic, and the northern region of Italy joined the EISS. On a website on the internet - http://www.eiss.org -, EISS continuously displays a summary of the influenza situation in Europe. This summary is updated weekly. Reports are also published regularly in the literature on the results of the Scheme (19-21).

1.5 Reporting the data observed in the NIVEL/RIVM surveillance of ARI

The results of the examinations in the framework of the NIVEL/RIVM surveillance of ARI are reported directly to the mailing GPs. During the epidemic period, they are weekly summarized in the form of tables in the "Nieuwsbrief Influenza Surveillance" ("Newsletter Influenza Surveillance"). This is a collaborative publication of the NIC (National Influenza Centre of the Netherlands, which is a collaboration of the RIVM and the Erasmus University Rotterdam, EUR), the NIVEL, and the IGZ (Inspectorate of the Health Care of the Netherlands).

The NIVEL/RIVM surveillance of ARI has been the subject of annual RIVM reports (15,16). In addition, the Dutch influenza epidemics and the antigenic and molecular characteristics have been described in other reports (latest in 1997, ref. 17) and in the February reports to the WHO in aid of their annual update of the influenza vaccine recommendation. The obtained data have also been published annually in the Nederlands Tijdschrift voor Geneeskunde (Dutch Journal for Medicine) (22). At the symposium of the European Society for Clinical Virology in Hamburg (30 August - 2 September 1998), a poster was presented describing the results of the NIVEL/RIVM

surveillance of ARI in the 1996/97 season (ref. 23 and Appendix 1). All publications mentioned in this paragraph were prepared in collaboration with EUR within the framework of the NIC, and with the NIVEL.

2. MATERIALS AND METHODS

2.1. Standard operating procedure (SOP) for taking nose/throat swabs from respiratory patients for the NIVEL/RIVM surveillance of ARI

The RIVM provides the participating GPs with gelatin-lactalbumin-yeast (GLY) virus transport medium (24) in tubes, sterile wooden sticks with cotton wool at the tip, and a preprinted questionnaire for the particulars of the patient. The GLY virus transport medium contains 200μg gentamicin and 100μg pimaricin per ml and is stored by the GP at +4°C until use.

The sampling procedure is as follows:
1. The GP inserts the cotton wool deep in the nose and passes it over the mucous membrane of the septum and the conchae.
2. He immerses the cotton wool in the transport medium in the tube, breaks the wooden stick so that the tube can be closed.
3. A second swab is passed over the pharynx arches and the posteral wall of the oropharynx, and immersed in the same tube as the nose swab. This wooden stick is also broken.
4. The GP fills out the form containing questions about the patient and his/her illness, see Appendix 2.
5. The GP mails the swabs and the questionnaire to the RIVM on the same day, or, if this is not possible, stores the swabs at +4°C until dispatch. Separate swabs were taken for the bacteriological examinations, see under section 2.3.

2.2 Outline of the virological examinations

If possible, the clinical sample was inoculated on cell cultures on the day of receipt at the RIVM. If this was not feasible, the specimen was stored at +4°C. Virus isolation was performed according to standard methods (25, 26). Four cell cultures were inoculated: tertiary cynomolgus monkey cells (tMK), human diploid lung fibroblasts (local cell strain GaBi), HEp-2 cell line, and R-HeLa cell line (a rhinovirus-sensitive subline of HeLa). After inoculation, tMK cells were incubated in the presence of trypsin. All cell cultures were incubated in roller drums at 33°C. Samples were also inoculated onto tMK and diploid cell cultures in flat-bottom tubes and centrifuged at 5000xg at room temperature for 75 minutes before stationary incubation at 33°C.

The influenza viruses were (sub)typed in haemagglutination inhibition assays using turkey erythrocytes (17, 26, 27). Other viruses were also identified by standard methods

(25, 26). In the 1996/97 season, PCR detection methods for seven pathogens were used in conjunction with virus isolation. Published primers were used for RS virus (28), coronaviruses OC43 and 229E (29), *Mycoplasma pneumoniae* (Mp) (30), and *Chlamydia pneumoniae* (Cp) (31). Rhinoviruses were detected using a PCR technique which was newly developed at the RIVM for the purpose of the NIVEL/RIVM surveillance of ARI (32).

2.3 Outline of the bacteriological examinations

Separate swabs for bacteriological examination were taken from 159 patients, who were also virologically sampled at the same visit. The swabs were passed over the oropharyngeal area only, put into bacteriological transport medium, and mailed directly to the bacteriological laboratory at the St Elisabeth Hospital in Tilburg (head: Dr M.F. Peeters). At Tilburg, the bacteriological specimens were cultured for conventional bacteria according to standard methods (33). The swabs were inoculated onto two 5% sheep blood agar plates and one chocolate agar plate. One blood agar plate was incubated at 35°C aerobically and one anaerobically. The chocolate agar was incubated in 5% CO_2 at 35°C. Since there are no hard and fast rules that divide micro-organisms into clear-cut categories of harmless commensal organisms and pathogenic species, isolation of so-called pathogenic species in low numbers was considered of no significance.

3. RESULTS AND DISCUSSION

3.1 The course of the 1996/97 respiratory season in the Netherlands

The 1996/97 respiratory season started in September, with an increase in the proportion of patients in whom an infection with a virus, Mp, or Cp could be established (Fig. 3). Most of the infections were due to rhinoviruses. This phenomenon was also observed in the 1995/96 season (16). A yearly increase in all respiratory illnesses was also reported in the countries surrounding the Netherlands during the same period (data EISS, see 1.4; an example is given in Appendix 3). This proportional rise in detectable infections in our country probably represents a real September wave of respiratory infections. This increase coincides with the commencement of a new school year, an event with which it may be causally related. When the new school classes are formed, there is ample opportunity for viruses originating from various families and holiday resorts to explore new hosts. Total respiratory diseases are not registered in the Netherlands. Therefore, it is not possible to verify the existence of such a wave in this country.

The proportion of positive samples declined in October, November, and the first half of December and rose again in the second half of December with the start of the annual influenza epidemic. This epidemic appeared at the usual time and had the usual length, course, and severity (Fig. 4). The peak of the number of influenza virus isolates was in weeks 3 and 4 and that of the incidence of ILI in weeks 4 and 5 of 1998 (Fig. 5). The epidemic began with a major wave of subtype A(H3N2) virus infections, followed by a small overlapping wave of type B virus infections in weeks 4 - 9; a single subtype A(H1N1) virus strain was isolated in week 8 by a diagnostic laboratory. Until week 6, the number of influenza virus isolates from the NIVEL/RIVM surveillance was smaller than that from the (mostly hospital-based) diagnostic laboratories as analysed at EUR (Fig. 6). During weeks 7 and 8, however, this ratio reversed. The reversion coincided with a small shoulder in the ILI curve and a small peak in the wave of type B virus infections (Fig. 5). The difference in the A(H3N2) : B virus isolation ratio between the GP and the hospital circuits is more clearly visible when "pies" are constructed for the whole season. Sixty-one percent of the influenza viruses isolated from GP patients were H3N2, while 88% of those isolated in diagnostic laboratories were this subtype (Fig. 7). This phenomenon probably reflects the higher pathogenicity of subtype A(H3N2) compared with type B and has been described earlier (18). In fact, this phenomenon recurs annually (Fig. 8 and Table 1).

In the 1995/96 season, a second increase in influenza activity was observed at the tail of the influenza epidemic (16). This period was characterized by a significant rise in the

proportion of (H1N1 and B) influenza virus-positive samples from the NIVEL/RIVM surveillance, which was as high as otherwise seen only during the peak of an influenza epidemic. Remarkably, this "wave" was not reflected in an enhanced rate of ILI. The phenomenon did not occur during the 1996/97 season.

Table 1. Influenza viruses isolated in the Netherlands 1991-1997

Season	Origin of strains	Total	H3N2	H1N1	B	A(H3N2)/B
1991/92	NIVEL/RIVM GP pilot study	10	7	3	0	>7
	virological laboratory RIVM *	251	184	67	0	>184
1992/93	NIVEL/RIVM GP surveillance	68	6	4	58	0.10
	diagnostic Dutch laboratories **	148	45	5	98	0.46
1993/94	NIVEL/RIVM GP surveillance	67	67	0	0	>67
	diagnostic Dutch laboratories **	197	196	0	1	196
1994/95	NIVEL/RIVM GP surveillance	56	15	1	40	0.38
	diagnostic Dutch laboratories **	146	74	1	71	1.04
1995/96	NIVEL/RIVM GP surveillance	121	81	17	23	3.5
	diagnostic Dutch laboratories **	335	245	50	40	6.1
1996/97	NIVEL/RIVM GP surveillance	127	78	0	49	1.6
	diagnostic Dutch laboratories **	185	161	1	23	7.0
1997/98	NIVEL/RIVM GP surveillance	82	75	1	6	12
	diagnostic Dutch laboratories **	299	297	2	0	>297

* Numbers of isolates from RIVM and other Dutch laboratories examined at RIVM.
** Numbers of isolates from Dutch laboratories except RIVM, examined at EUR.

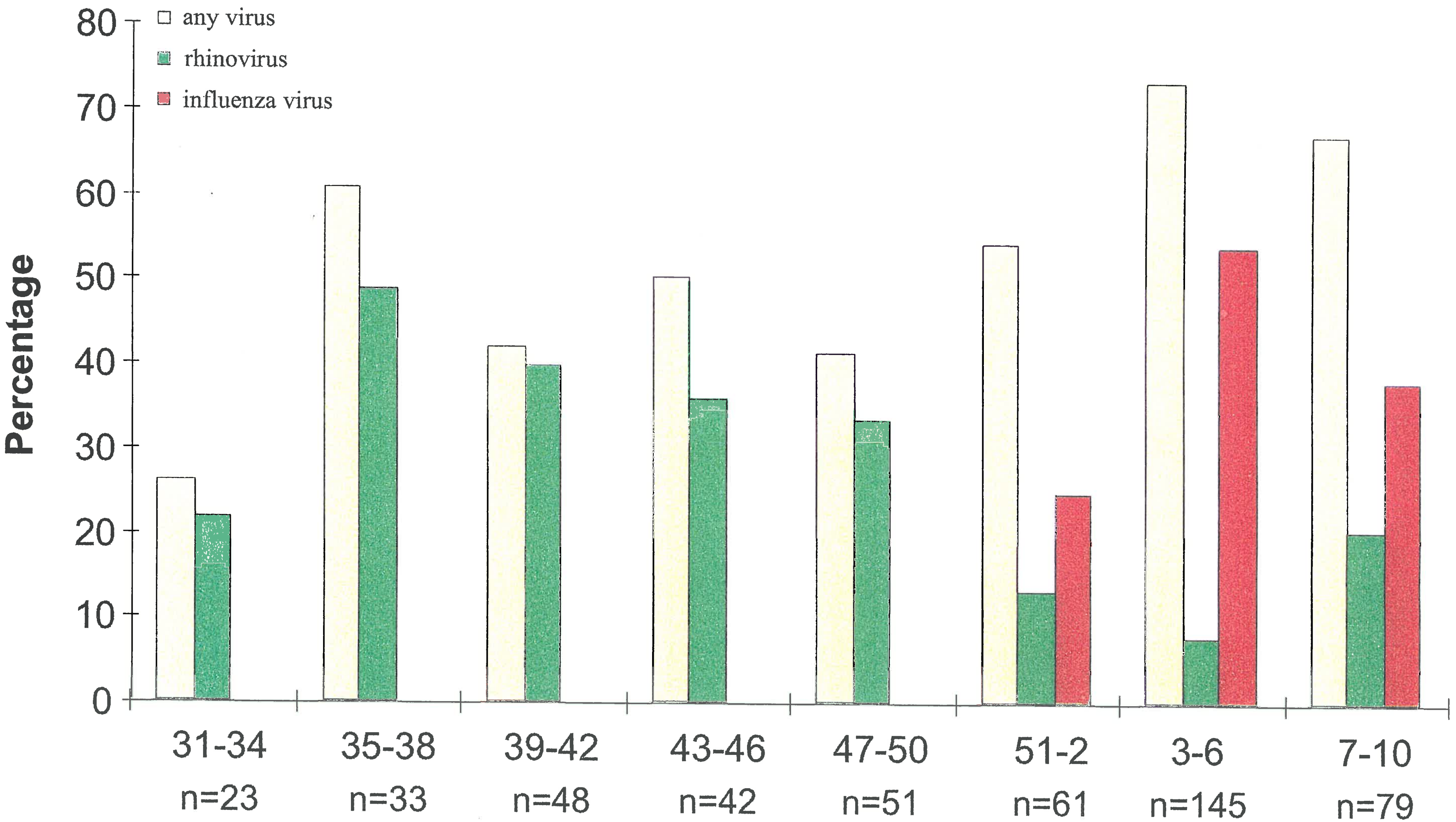


Figure 3. Respiratory 1996/97 season. Percentages of samples positive for any virus, rhinovirus, and influenza virus, respectively, per 4 weeks

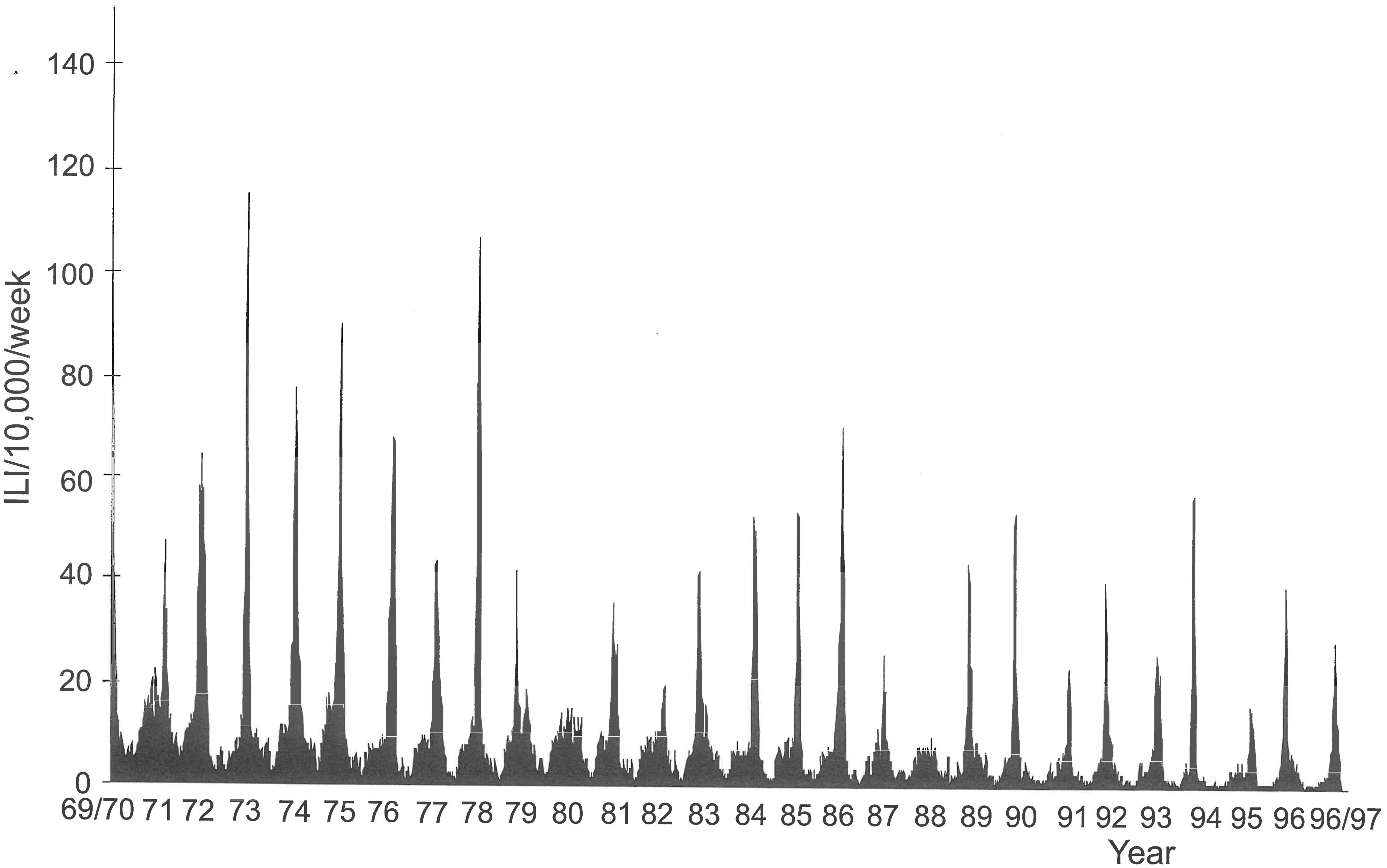


Figure 4. Influenza-like illnesses (ILI) per 10,000 inhabitants per week among patients presenting in general practices in the Netherlands (Source: NIVEL)

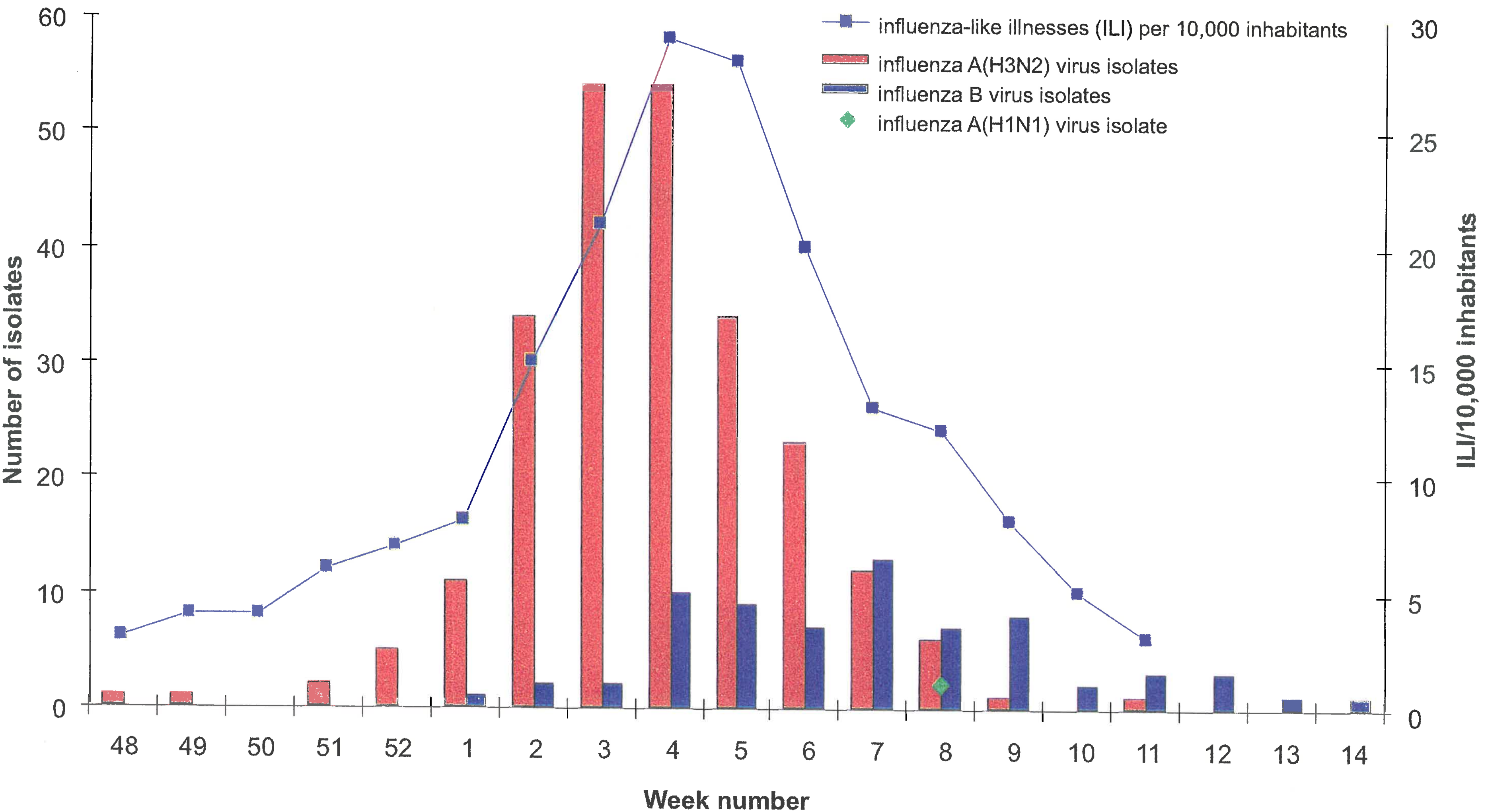


Figure 5. Influenza epidemic in the Netherlands in the 1996/97 season: clinical incidence and virus isolates by (sub)type

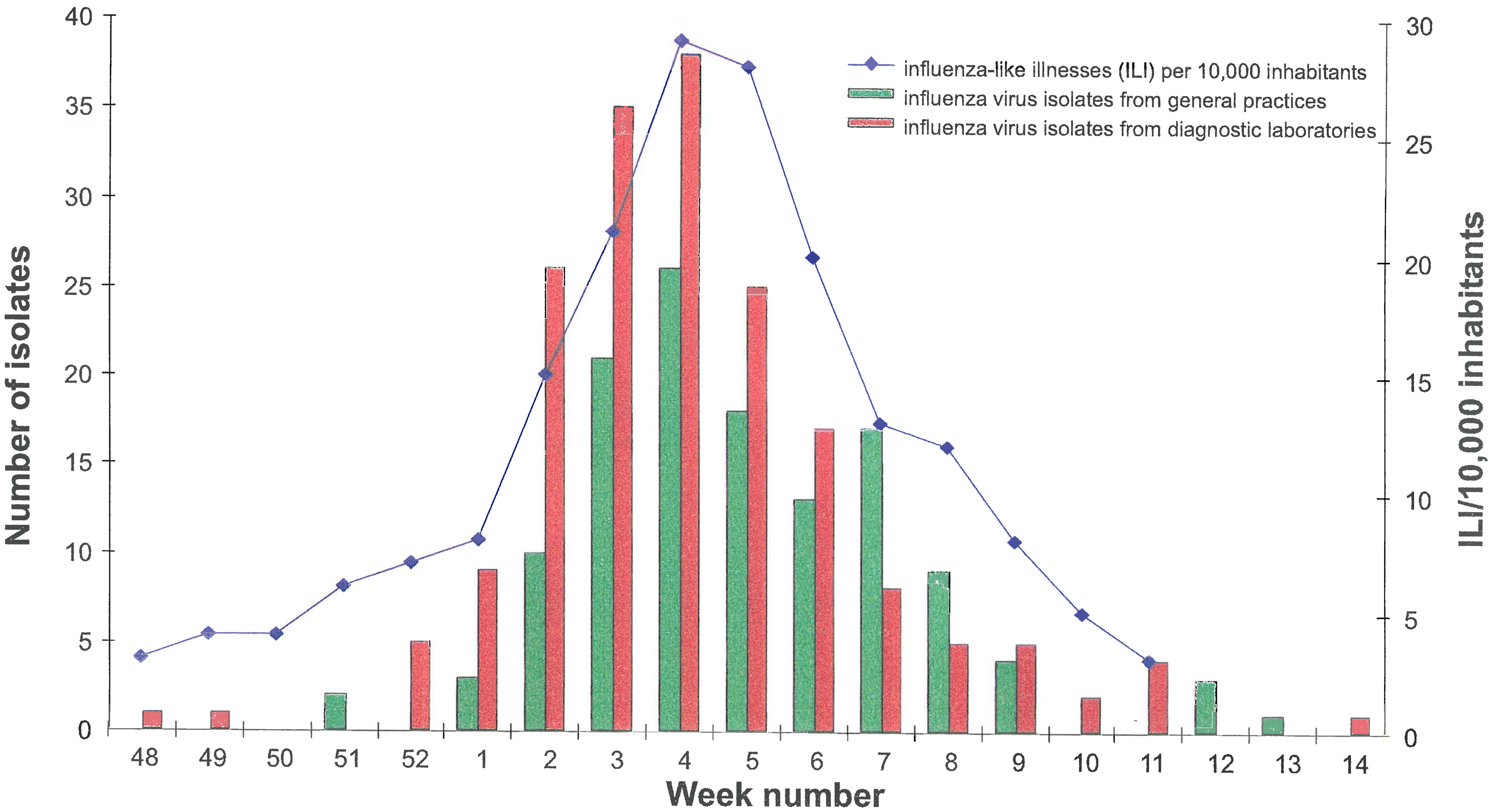
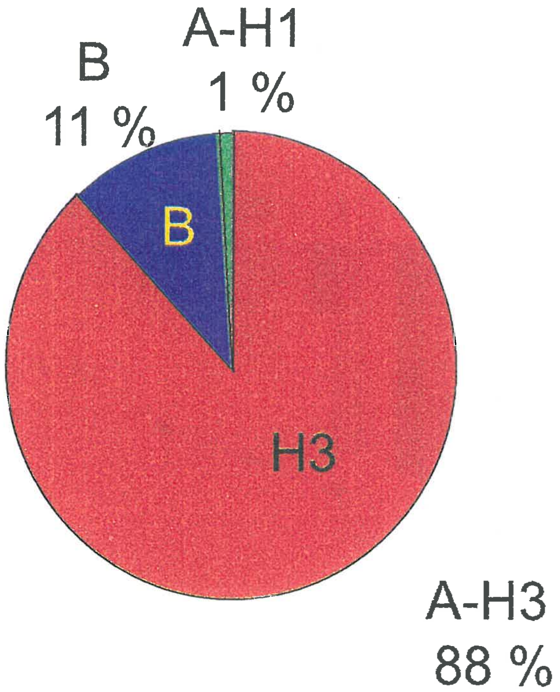


Figure 6. Influenza epidemic in the Netherlands in the 1996/97 season: clinical incidence and virus isolates by origin



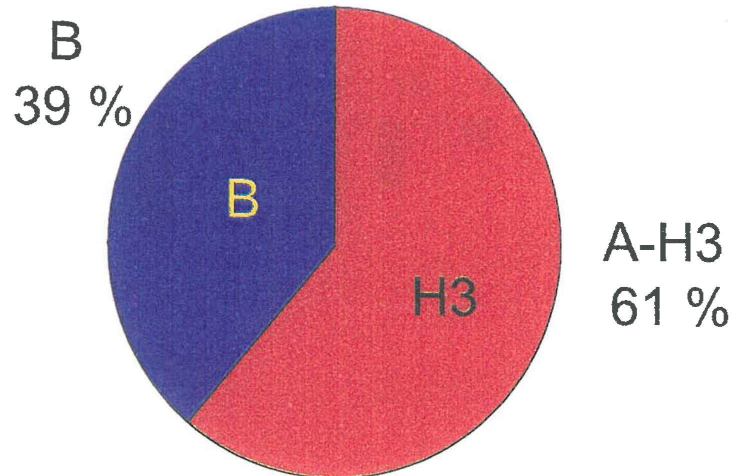


Figure 7. Influenza virus isolates from diagnostic (hospital-based) laboratories versus general practices (GP) in the 1996/97 season

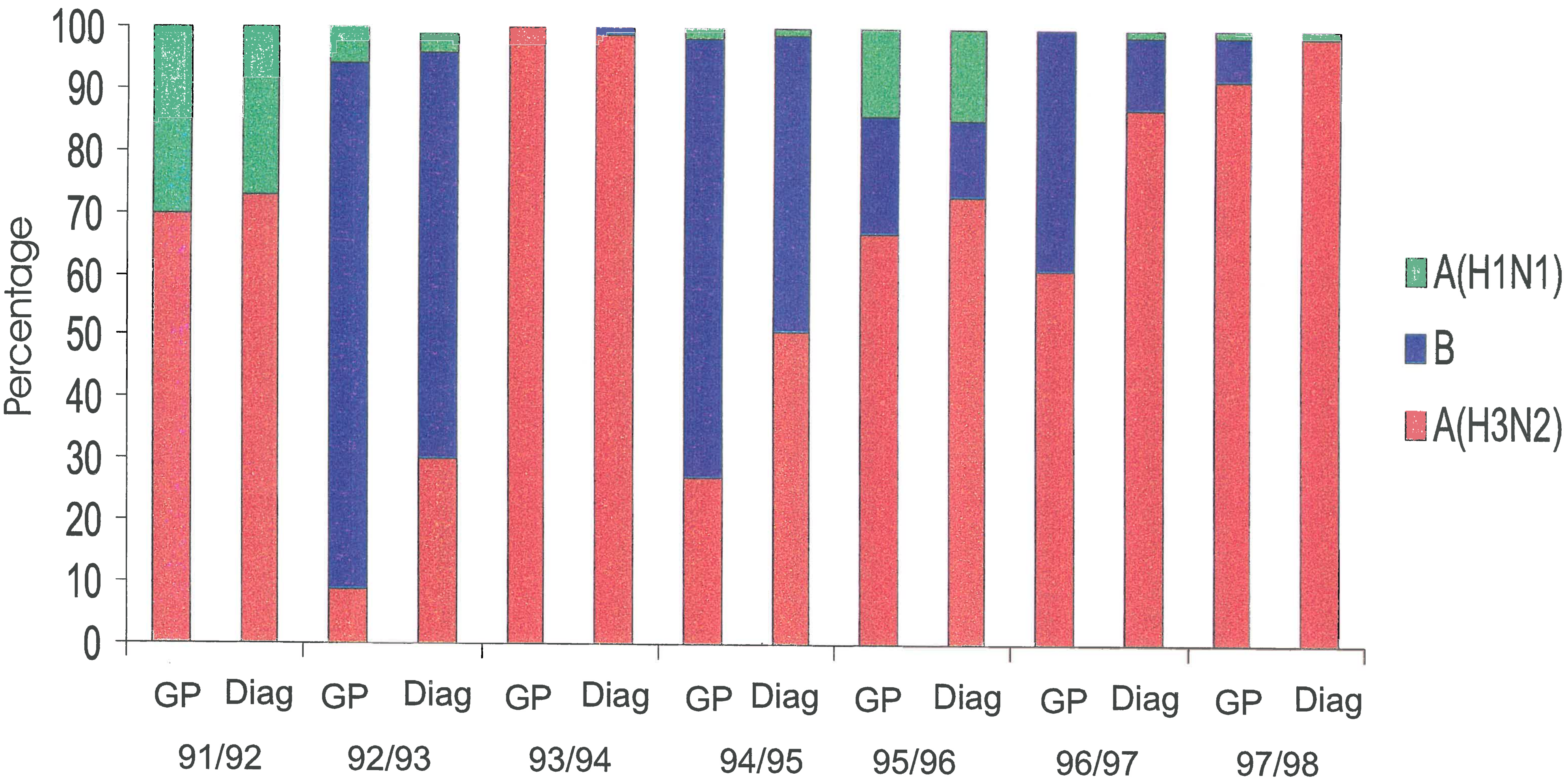


Figure 8. Influenza virus isolates from general practices (GP) versus diagnostic (hospital-based) laboratories (Diag) in the 1991/92-1997/98 seasons

3.2 Detections of viruses and bacteria: the numbers

In the period from week 29 of 1996 up to and including week 28 of 1997, we received in total 540 clinical samples from 540 ARI patients from 36 of the 43 participating sentinel stations of NIVEL. A summary of the results of all examinations for viruses and bacteria using culture and PCR is presented in Table 2 and Figure 9. In the upper part of the table, we present the numbers and percentages of patients positive for the specified viruses, Mp, or Cp. In the lower part of the table, one can see the corresponding data for conventional bacteria. Overall, we found infections with an identified pathogen in 63.6 % of the patients; in this report the term "pathogen" includes all viruses and bacteria, conventional or fastidious like Mp and Cp, that are potentially pathogenic for the respiratory tract. After correction for patients with prolonged shedding (see section 3.4), 60.9% of the patients were found to have had an identified infection simultaneously with the presence of respiratory symptoms.

Either a potentially pathogenic virus/Mp/Cp was demonstrated in 296 (54.8%) of the 540 patients (Table 2). We detected herpes simplex virus (HSV) in 18 of the 540 patients, in 10 of whom it was the only pathogen. No HSV was noted in Table 2, however, because the presence of HSV is generally the consequence of the reactivation of a lifelong latent HSV infection. Such an event supposedly causes respiratory disease only in immunocompromised individuals, who were unlikely to have been included in the present study. Correction for prolonged shedders (see section 3.5) shows that 53.4% of the patients had a virus/Mp/Cp that was potentially pathogenic for the airways and that the infection coincided with their respiratory disease.

The numbers of samples provided and the rates of virus/bacterium detection differed considerably between the various sentinel stations (Table 3). In the 1996/97 season, the total number of specimens varied between zero (seven stations) and 69, median 9.5. The percentage of pathogen-positive samples for stations from which at least ten specimens were received ranged from 31% to 82%, median 58%.

As mentioned above, the detections of the various pathogens were not evenly distributed over time (Table 4). Rhinovirus detections peaked in September and early December, RS virus in late December and mid-January, influenza A(H3N2) virus in mid-January, and influenza B virus in mid-February.

For the first time since the surveillance started in 1991/92, we examined patients for the presence of potentially pathogenic conventional respiratory bacteria. The bacterium species that could be detected in these examinations are listed in the lower part of Table 2. Because of organizational and financial difficulties this bacteriological study started later than the virological surveillance and was performed with the help of five of the 44

sentinel stations from week 45 of 1996 up to and including week 28 of 1997. One or two bacterium species were grown from the samples from 26 (16.4%) of the 159 examined patients (Table 2); 12 samples were from ILI-positive and 14 from ILI-negative patients. *Proteus mirabilis* was not considered pathogenic and was not noted on the table for the same reason that led to the exclusion of HSV, see above. In 12 (7.6%) of the 26 bacterium-positive patients also a virus, Mp, or Cp could be detected. In 14 (8.8%) of these 26 patients no viruses, Mp or Cp were detected. Corrected for prolonged shedding (see section 3.5), 12 (7.5%) of the patients had a conventional respiratory bacterium as the only detected potentially pathogenic pathogen.

Table 2. Summary of the results of virus/bacterium culture and -PCR of the NIVEL/RIVM GP surveillance of respiratory "viral" illnesses in the 1996/97 season (15 July 1996 - 13 July 1997)

Respiratory pathogen	**Positive:** culture	**Positive:** PCR	**follow-up samples**
540 virological specimens: 15 July 1996 - 13 July 1997			
influenza A(H3N2) virus	78 (14.4%)		
influenza B virus	49 (9.1%)		
parainfluenza virus	5 (0.9%)		
RS virus	4 (0.7%)	29 (5.4%)	
rhinovirus	22 (4.1%)	119 (22.0%)	
enterovirus	3 (0.6%)	21 (3.9%)	
adenovirus	6 (1.1%)		
coronavirus OC43		9 (1.7%)	
coronavirus 229E		0	
Mycoplasma pneumoniae		7 (1.3%)	
Chlamydia pneumoniae		6 (1.1%)	
Culture-positive samples	**167 (30.9%)**		
PCR-positive samples	**191 (35.4%)**		**still PCR-positive $:**
Total culture- and/or PCR-positive	**296 (54.8%)** #		**1/25 (4%)** (RS virus)
Idem, corrected for long shedders	**284 (53.4%)**		
159 bacteriological specimens: 5 November 1996 - 13 July 1997 (all 159 concerning patients were also virologically examined)			
Streptococcus haemolyticus A	7 (4.4%)		
Streptococcus haemolyticus B	3 (1.9%)		
Streptococcus haemolyticus C	1 (0.6%)		
Streptococcus haemolyticus F	1 (0.6%)		
Streptococcus haemolyticus G	1 (0.6%)		
Streptococcus pneumoniae	2 (1.3%)		
Haemophilus influenzae	5 (3.1%)		
Haemophilus parainfluenzae	3 (1.9%)		
Staphylococcus aureus	2 (1.3%)		
Enterobacter	2 (1.3%)		
Escherichia coli	1 (0.6%)		
Moraxella catarrhalis	0		
Bacterial infections	**28**		**still bact. positive $:**
Bacterium-positive samples	**26 (16.4%)**		**1/8 (12%)** @
Bacterium-positive/virus-negative samples	**14 (8.8%)**		(*Streptococcus*
Idem, corrected for long shedders	**12 (7.5%)**		*haemolyticus* A)

Total VIR - and/or BAC - positive samples: 63.6%

Total VIR - and/or BAC - positive samples, corrected for long shedders: 60.9%

$ After on an average 30 days (VIR) and six weeks (BAC) one recovered patient was still positive.

In 1994/95 season: **35%**.

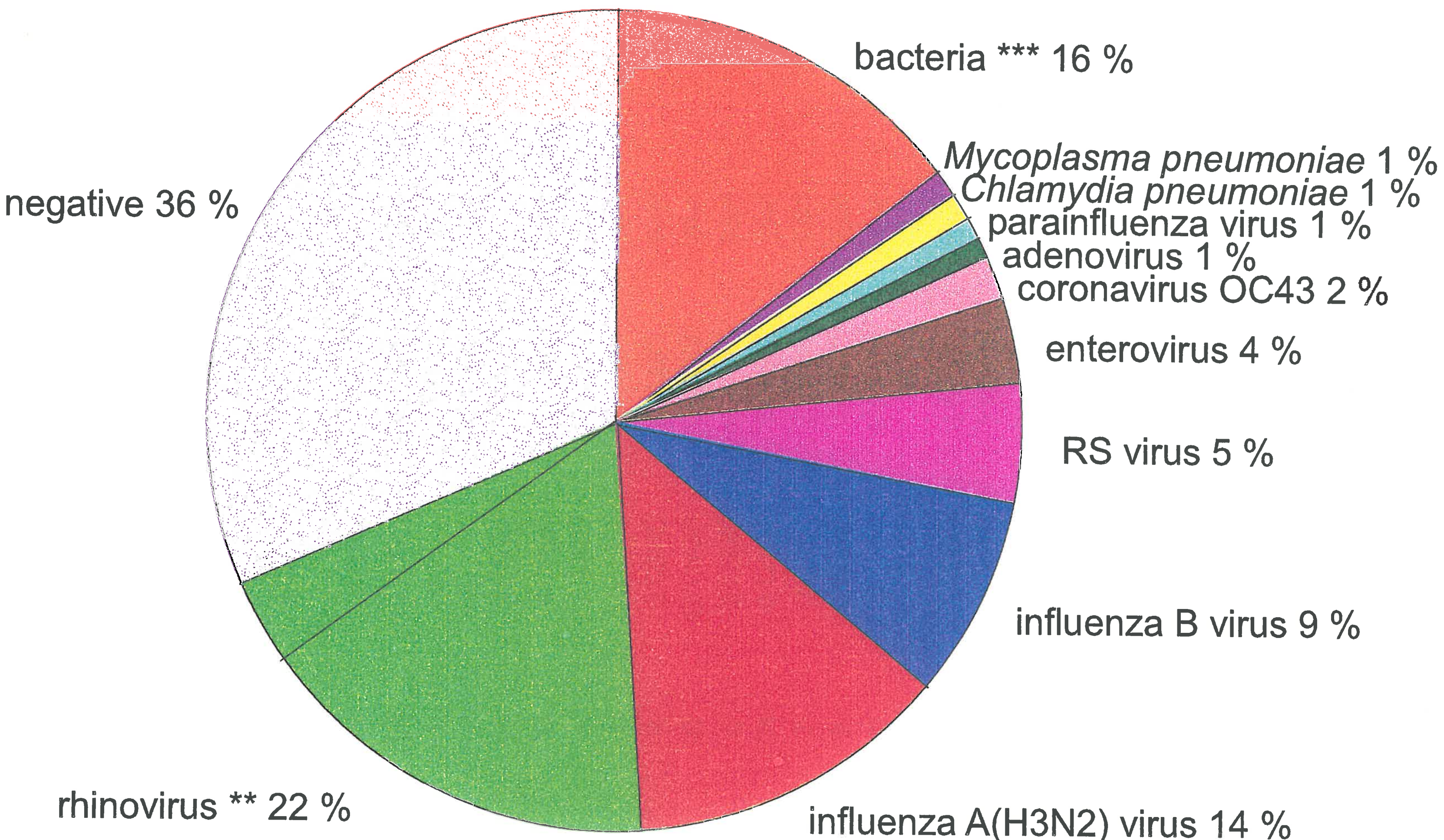


Figure 9. Percentages of viruses and bacteria detected in 540 respiratory GP patients (NIVEL) in the Netherlands in the 1996/97 season (culture and PCR)*

* Results are given as percentage of samples positive for the agent (sum is more than 100%)

** Small sector: culture- and PCR-positive (4%), whole sector: PCR-positive (22%)

*** Bacterial examination was done with 159 of the 540 patients

Table 3. Results of the NIVEL/RIVM surveillance per sentinel station during the 1996/97 season

sentinel station no	region	urbanization	no of samples	influenza-positive	other pathogens	% positive
1	north	city	4	2 (15%)	1 (25%)	75%
2	north	village	27	4 (15%)	11 (41%)	56%
3	north	town	9	2 (22%)	5 (56%)	78%
4	north	village	16	6 (38%)	4 (25%)	62%
5	east	town	7	3 (43%)	2 (29%)	71%
6	east	town	10	4 (40%)	3 (30%)	70%
7	east	town	17	5 (29%)	9 (53%)	82%
8	east	village	12	4 (33%)	1 (8%)	42%
9	east	town	1	0	0	0%
10	east	city	8	0	0	0%
11	east	city	13	6 (46%)	2 (15%)	62%
12	east	town	3	1 (33%)	1 (33%)	67%
13	west	city	1	0	1(100%)	100%
14	west	city	6	1 (17%)	1 (17%)	33%
15	west	town	3	0	1 (33%)	33%
16	west	city	24	5 (21%)	8 (33%)	54%
17	west	town	7	2 (29%)	0	29%
18	west	city	42	15 (36%)	10 (24%)	60%
19	west	town	6	3 (50%)	1 (17%)	67%
20	west	town	1	0	1(100%)	100%
21	west	town	4	1 (25%)	2 (50%)	75%
22	west	city	23	6 (26%)	9 (39%)	65%
23	west	city	31	9 (29%)	4 (13%)	42%
24	west	city	1	0	1(100%)	100%
25	west	town	2	0	1 (50%)	50%
26	west	city	24	11 (46%)	6 (25%)	71%
27	west	town	18	1 (6%)	5 (28%)	33%
28	south	town	69	8 (12%)	24 (35%)	46%
29	south	town	6	2 (33%)	2 (33%)	67%
30	south	town	11	6 (55%)	0	55%
31	south	town	18	4 (22%)	9 (50%)	72%
32	south	town	2	0	1 (50%)	50%
33	south	town	13	4 (31%)	0	31%
34	south	town	62	6 (10%)	32 (52%)	61%
35	south	city	3	0	0	0%
36	south	city	35	5 (14%)	12 (34%)	49%

Table 4. Detection of respiratory viruses, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* in 540 nose/throat swabs from 540 patients in the 1996/97 season using virus isolation and PCR arranged according to week and pathogen (herpes simplex virus isolates not included). Blank entry means no detections

Week	No of samples	positive samples	influenza A(H3N2)	influenza A(H1N1)	influenza B	rhinovirus	entero virus	corona virus[1)]	RS virus	para influenza	adeno virus	Mycopl pneu	Chlam pneu	Double infections	Triple infections
29/30	2	1 (50%)										1			
31/32	14	3 (21%)				3									
33/34	9	3 (33%)				2	1								
35/36	14	6 (43%)				6					1			1x	
37/38	19	13 (68%)				10	2			1					
39/40	20	10 (50%)				11				1				1x	
41/42	28	8 (29%)				8						1		1x	
43/44	21	12 (57%)				8	2		1		1				
45/46	21	10 (48%)				7			2		1				
47/48	18	9 (50%)				6	1		1	1					
49/50	32	13 (41%)				11		3	1					1x	
51/52	20	13 (65%)	2			6		1	5			1	1	3x	
1/2	41	19 (46%)	12		1	4			1	1			2	2x	
3/4	86	61 (71%)	38		9	7	6	1	7				2	8x	1x
5/6	59	39 (66%)	18		13	6			3			1		2x	
7/8	51	34 (67%)	8		18	7	1	1	3				1	2x	2x
9/10	30	16 (53%)			4	5	2	1	2		2	1		1x	
11/12	13	7 (54%)			3		1	1	1	1					
13/14	8	8 (100%)			1	6	2	1			1			3x	
15/16	9	5 (56%)				2	2		1			1		1x	
17/18	5	2 (40%)				2			1					1x	
19/28	20	4 (20%)				2	1					1			
Total	540	296 (55%)	78		49	119	21	9	29	5	6	7	6	27x	3x

1) All detected coronaviruses were of type OC43 and no type 229E viruses were observed.

Table 5. Isolation of conventional potentially pathogenic bacteria from 159 nose/throat swabs from 159 patients in the NIVEL/RIVM surveillance of respiratory virus infections in the 1996/97 season arranged according to week and bacterium species 1)

Week	Number of samples	Positive samples	Positive samples - virus 3)	*Strep. haem.* 2)	*Strep. pneu.*	*Haem. (para) influ* 4)	*E. coli*	*Staph. aureus*	*Entero-bacter*	Double bacterial infections	Virus, *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* in the same samples
45/46	11	3 (27%)	*2*	1		2	1			1	adenovirus
47/48	9	1 (11%)	*0*		1						rhinovirus
49/50	14	3 (21%)	*2*	2		1					rhinovirus, coronavirus OC43
51/52	6	1 (17%)	*1*	1							
1/2	15	3 (20%)	*2*	1		2					rhinovirus
3/4	27	0	*0*								
5/6	12	4 (33%)	*2*	3				1			rhinovirus (in 2 samples)
7/8	19	3 (16%)	*2*	1		2		1		1	influenza B virus
9/10	14	3 (21%)	*1*	2	1						rhinovirus and influ. B virus (2x)
11/12	7	3 (43%)	*3*			1			2		
13/14	4	1 (25%)	*0*	1							rhinovirus
15/16	5	0	*0*								
17/18	2	0	*0*								
19/28	14	1 (7%)	*0*	1							Mycoplasma pneumoniae
Total	**159**	**26 (16%)**	**15 (9%)**	**13 ***	**2**	**8 ****	**1**	**2**	**2**	**2**	

1) The culture of *Proteus mirabilis* is not included in this table because it is not considered a respiratory pathogen. A blank entry means negative culture.
2) *Streptococcus haemolyticus* group A: 7; group B: 3; group C: 1; group F: 1; group G: 1.
3) Number of positive bacterial samples in which no virus, *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* was detected.
4) *Haemophilus influenzae* : 5; *Haemophilus parainfluenzae*: 3.

3.3 Age dependency of virus and bacterium detection

Roughly, the age distribution of the samples provided for virological examination1 reflected the age distribution of the general population, except that the youngest were over-represented and the elderly under-represented (Table 6). The group of 0-4-year-old children not only was over-represented but also yielded the highest rate (71%) of detections of any virus/Mp/Cp. Both phenomena could be explained by the limited spectrum of specific immunities in this age class because of the low number of previous infections and to the relatively low level of hygiene and a high frequency of intimate contacts with other children. The situation of the elderly appears to mirror that of the 0-4-year-old children. They were under-represented in the samples and yielded the lowest rate (42%) of detections of any virus/Mp/Cp (Table 6). Also the explanations may be similar. Elderly have acquired an elaborate range of specific immunities during their lives and have relatively few contacts with small children. Our "mirror" findings with the youngest and the oldest individuals agree with those of Monto and Sullivan, who observed the same trends (1).

Table 6. Detection of viruses, Mp, and Cp in the 1996/97 season arranged according to age group

Age group (yrs)	Dutch population on 1-1-96 (%)[1]	Samples examined (%)	Samples with pathogen (%)	Multiple infections (%)
0-4	981,000 (6%)	58 (11%)	41 (71%)	10 (17%)
5-14	1,867,000 (12%)	37 (7%)	23 (62%)	3 (8%)
15-44	7,015,000 (45%)	268 (50%)	143 (53%)	11 (4%)
45-64	3,570,000 (23%)	119 (22%)	62 (52%)	6 (5%)
>64	2,061,000 (13%)	45 (8%)	19 (42%)	0 (0%)
unknown		13 (2%)	8 (61%)	0 (0%)
Total	15,494,000 (100%)	540 (100%)	296 (55%)	30 (6%)

1) The data on the Dutch population were supplied by Statistics Netherlands (CBS).

The numbers of cultured conventional bacteria were too small to sort out the age dependency (Table 7).

Table 7. Detection of conventional bacteria in the 1996/97 season arranged according to age group

Age group (yrs)	Dutch population on 1-1-96 (%)	Samples examined (%)	Samples with pathogen (%)	Multiple infections (%)	Samples with conventional bacterium only (%
0-4	981,000 (6%)	15 (10%)	2 (13%)	1 (7%)	1 (7%)
5-14	1,867,000 (12%)	16 (10%)	5 (31%)	4 (25%)	1 (7%)
15-44	7,015,000 (45%)	79 (51%)	13 (16%)	8 (10%)	5 (6%)
45-64	3,570,000 (23%)	28 (18%)	2 (7%)	0 (0%)	2 (7%)
>64	2,061,000 (13%)	15 (10%)	3 (20%)	1 (7%)	2 (13%)
unknown		1 (1%)	0 (0%)	0 (0%)	0 (0%)
Total	15,494,000 (100%)	154 (100%)	25 (16%)	14 (9%)	11 (7%)

3.4 Multiple infections

Double and triple infections with virus/Mp/Cp were detected in 27 (5.0%) and 3 (0.6%) of the 540 samples, respectively, during the 1996/97 season (Table 4). Table 8 presents the numbers of multiple infections that were observed with each of the various virus/Mp/Cp, cumulated for the two seasons in which PCR detection was applied, namely the 1994/95 and 1996/97 seasons. Because no PCR detection was applied in the other three seasons, the rates of multiple infections were much lower in those years.

Table 8. Multiple infections with virus/Mp/Cp in the 1994/95 and 1996/97 seasons

Respiratory pathogen	Number (% of total of 1097)	Double infections (% of positives)	Double and triple infections (%)
influenza A(H3N2) virus	96 (9%)	10 (10%)	11 (11%)
influenza A(H1N1) virus	1(0.1%)	1 (100%)	1 (100%)
influenza B virus	101 (9%)	3 (3%)	5 (5%)
rhinovirus	170(15%)	17 (10%)	19 (11%)
RS virus	45 (4%)	13 (29%)	14 (31%)
enterovirus	27 (1%)	7 (26%)	8 (30%)
parainfluenzavirus	7 (1%)	1 (14%)	1 (14%)
adenovirus	19 (2%)	3 (16%)	3 (16%)
coronavirus	34 (3%)	4 (12%)	5 (15%)
Mycoplasma pneumoniae	14 (1%)	5 (36%)	5 (36%)
Chlamydia pneumoniae	12 (1%)	6 (50%)	7 (58%)
Total number of infections	526*	70*	79*

* Two respiratory pathogens were demonstrated in 35 clinical samples and three respiratory pathogens were found in three samples.

Table 9 presents the occurrence of multiple infections analysed according to various combinations of virus/Mp/Cp, again for the same two seasons. Two conventional bacterium species were grown from each of two (1.5%) of the 159 patients. These were omitted from Tables 8 and 9 because we examined conventional bacteria in only 159 of the 540 samples from the 1996/97 season and in none of the 557 samples from the 1994/95 season. The total number of detected bacterial infections was too small anyway to allow conclusions about specific combinations of double infections.

Table 9. Double and triple infections with combinations of virus/Mp/Cp in the 1994/95 and 1996/97 seasons

Combination of pathogens			Numbers of samples
rhinovirus	*Mycoplasma pneumoniae*		2
rhinovirus	adenovirus		2
rhinovirus	parainfluenzavirus		1
rhinovirus	RS virus		5
rhinovirus	influenza A(H3N2) virus		1
rhinovirus	enterovirus		2
rhinovirus	coronavirus OC43		3
influenza A(H3N2) virus	*Chlamydia pneumoniae*		4
influenza A(H3N2) virus	RS virus		4
influenza A(H3N2) virus	enterovirus		2
influenza A(H1N1) virus	*Chlamydia pneumoniae*		1
influenza B virus	*Chlamydia pneumoniae*		1
Mycoplasma pneumoniae	enterovirus		1
RS virus	adenovirus		1
RS virus	coronavirus OC43		1
RS virus	*Mycoplasma pneumoniae*		2
nfluenza B virus	enterovirus		2
hinovirus	influenza A(H3N2) virus	enterovirus	1
hinovirus	influenza B virus	coronavirus OC43	1
RS virus	influenza B virus	*Chlamydia pneumoniae*	1

On an average, 16% of the infections in the 1994/95 and 1996/97 seasons were either double or triple infections of virus/Mp/Cp. Relatively high percentages of double infections were observed for Cp and Mp (58 and 36%, respectively), and, to a lesser degree, for RS virus and enterovirus (31 and 30%, respectively). The total numbers for Cp and Mp are small, but the percentages still seem to be reliable because they were high in both seasons, namely 50% and 67% for Cp and 29% and 43% for Mp, respectively. Higher percentages of multiple infections with a particular pathogen may be explained in various ways, including a limited pathogenic potential. The high percentage (31%) involving RS virus may, therefore, be unexpected because infections with this virus can certainly lead to serious disease in babies. This sequel, however, occurs with only a small minority of babies and does not involve a GP in most cases.

Moreover, almost half of the RS virus infections observed in the present study were in 0-4-year-old children and almost three times as many multiple infections with any pathogen were found in this age class than in the average of all age groups (Table 6). This preponderance is probably related to the already mentioned and discussed observation that single infections also had the highest incidence in young children (Table 6, section 3.3).

3.5 Prolonged shedding: examination of follow-up samples

When considering the significance of the results of the present study for our knowledge of the aetiology of ARI in man, some general pathological principles of microbial infections should be kept in mind. Detection of a potentially pathogenic agent in a clinical sample does not prove that the pathogen caused the current disease in the patient concerned. The agent may be an "innocent bystander": a "commensal" pathogen, present for longer periods of time (months, years, life-long) that does not contribute to the current illness, or, alternatively, an agent causing a short-lived infection also without inducing any symptoms, in other words, a "subclinical" infection. In practice, it is impossible to know whether a detected pathogen is responsible for the current disease of the patient concerned.

Theoretically, a case-control study is the only way to estimate the percentage mentioned. The multitude of respiratory viruses, however, presents a serious problem for this approach. Not only different virus species, but also different serotypes and even different strains classified in the same serotype may differ widely in the fraction of subclinical infections. This degree of variation calls for large numbers of participants in the study and a diagnostic arsenal capable of identifying the detected viruses at least at the serotype level.

To avoid the matching problem and because a case-control study was too expensive, we addressed the question of the clinical relevance of the PCR detection of rhinovirus and RS virus in another way. We collected follow-up specimens from positive patients and re-examined them using the same PCR. In this way, each patient functioned as his own control and no matching of any kind was needed. The specimens were taken from 44 patients on an average of 31 days after the first sample. Of the 19 patients who still displayed respiratory symptoms, seven (37%) were still positive for the same pathogen that was detected in the first sample (Tables 10a and 10b).

Only one (4%) of the 25 recovered patients was still positive. Eight patients positive using bacterium culture were also resampled on an average of six weeks after the first sample. All of them had recovered, although one was still positive for the same bacterium (data not shown). This low rate of prolonged shedding means that, at least in

this population, long-term carriers occur infrequently and suggests a high association between disease and presence of the pathogen. Moreover, the percentages of the detected pathogens can be corrected for this phenomenon, as has been done in Table 2. Because of its small magnitude, the low accuracy of this correction is not of serious concern.

Table 10a. Rhinovirus detection in clinical follow-up samples from RT-PCR-positive patients

Follow-up samples from individuals[1)]	RT-PCR-positive	RT-PCR-negative	total
Still displaying respiratory disease	7	7	14
Healthy	0	19	19
total	7	26	33

1) Follow-up samples were taken on an average of 32 days after the first sample.

Table 10b. RS-virus detection in clinical follow-up samples from RT-PCR-positive patients

Follow-up samples from individuals[1)]	RT-PCR positive	RT-PCR negative	total
Still displaying respiratory disease	0	5	5
Healthy	1	5	6
total	1	10	11

1) Follow-up samples were taken on an average of 30 days after the first sample.

The examination of follow-up specimens does not, however, account for subclinical infections. There could have been a short-lived symptomless infection with the detected pathogen at the time of the first sample, whereas the examined disease really had another cause (infectious or non-infectious). In fact, the observation of double and triple infections with 15% of our virus/Mp/Cp-positive patients demonstrates that the simultaneous presence of different potentially pathological factors does occur. Correction for this type of fortuity can only be based on a well-designed case-control study.

3.6 Incidence of influenza-like illnesses (ILI) and influenza in the 1992/93 - 1996/97 seasons

As explained above, the numbers of ILI registered by the GPs participating in the NIVEL network differ from the number of influenza virus infections in the community. Many induce no or only minor symptoms, not severe enough to be called an ILI. Even when a real ILI follows, a high proportion of the patients does not consult their GP for various reasons. Monto and Sullivan estimated that only about one third of influenza patients in the community are seen by a GP (1); Govaert et al. (34) and Kerssens from NIVEL (35) found similar fractions. In contrast, the percentage of ILI that is actually caused by influenza viruses is certainly less than 100%, even when reported to a GP

and even in the peak weeks of the epidemic. Below, we will try to establish the fraction of ILI associated with an influenza virus infection and estimate the real incidence of influenza in the population of the Netherlands on the basis of the registered incidence of ILI, the "laboratory-based correction factor" (see below), and the fraction of influenza patients that consults a GP.

3.6.1 Incidence of influenza: the influenza correction factor

Our laboratory-based surveillance has shown that some of the ILI are not due to influenza virus infections. We obtained so-called influenza correction factors for all ILI and for specific subpopulations of the patients involved in the NIVEL/RIVM surveillance in the following manner.

Calculation of the estimated real influenza incidence in the Dutch community:

$$\text{Incidence}/10{,}000 = N_{(ILI/10{,}000)} \times \frac{7}{5} \times 3 \times \frac{N_i \text{ (samples from ILI with influenza virus)}}{N_a \text{ (all samples from ILI)}}$$

in which: $N_{(ILI/10,000)}$ = number of ILI/10,000 inhabitants of the specific group
7/5 = factor correcting for a 5 day workweek for GPs
3 = factor correcting for the number of patients with ILI who do not consult their GP (1, 33, 34)
N of numerator (N_i) = total number of ILI from which influenza virus was grown at the RIVM
N of denominator (N_a) = total number of virologically examined ILI
N_i / N_a = laboratory-based correction factor

Subsequently, we multiply the ILI incidences by the described factor 7/5 x 3 x N_i/N_a (= 1.1 for the period 1992/93 - 1996/97). In this way, we obtain what we could call the "estimated real influenza incidence in the Dutch community", abbreviated below to "**influenza incidence**". This figure is a minimum value. Undoubtedly, our methods for establishing an influenza virus infection are not 100% effective. In particular, serological and molecular methods and the addition of a PCR technique could enhance the detection rate. In fact, Ellis, Fleming, and Zambon devised a multiplex RT-PCR for influenza viruses A(H3N2), A(H1N1), and B, which in their surveillance increased the number of influenza virus-positive patients by 36% (36). The virus isolation procedure used in this study, however, did not include a centrifugation step during the virus adsorption phase (Ellis, personal communication). In our study, such a step enhanced the virus isolation rate by 42% (16). Perhaps, therefore, introduction of a centrifugation step may make the virus isolation method used in the present study as sensitive as the RT-PCR of Ellis et al. The fact that not all influenza virus culture-positive clinical specimens scored positive with the new multiplex PCR (36) suggests that more

sensitive PCR techniques are possible. Such techniques could increase the influenza virus detection rate.

3.6.2 Incidence of influenza: age dependency

Although influenza is typically a disease that strikes individuals of all ages, the incidence of reported ILI varies for different age groups (Table 11). Considering all five seasons studied, the highest incidence of ILI was found in the group of 0-4-year-old children, being almost twice the average incidence. The lowest ILI incidence was observed for the elderly group. When calculating the laboratory-based correction factors for various subpopulations, we found them to be markedly age-dependent (Table 12). The highest value for this correction factor (0.46) was that for the class of 5-14-year-old children. Using the influenza correction factor defined above, we subsequently calculated the estimated incidences of influenza as a function of age (Table 13). Remarkably, we found that in reality not the 0-4-year-old children but the 5-14-year-old children were the main target of the influenza epidemics in all five seasons studied. For them, the influenza incidence was about twice that of the average incidence for all ages.

Table 11. Incidence of ILI per 10,000 inhabitants registered by NIVEL from week 40 up to week 20 arranged according to age group and season

Age group (yrs)	1992/93	1993/94	1994/95	1995/96	1996/97	Mean of all seasons
0-4	502	581	261	430	320	419
5-14	424	328	143	261	203	272
15-44	280	242	197	230	206	231
45-64	225	285	183	254	221	234
>64	194	236	122	200	195	189
All ages	286	280	182	248	248	249

Table 12. Calculation of laboratory-based correction factor for influenza incidences in the period 1992/93 up to and including 1996/97 arranged according to age group

Age group (yrs)	Mean patient population NIVEL	Number of ILI reported to NIVEL [1]	Number of samples from ILI (%) [1]	Number of flu-positive samples (%) [1]	Lab-based correction factor
0-4	8,048	1686	100 (5.9%)	22 (22%)	0,22
5-14	16,911	2300	145 (6.3%)	66 (46%)	0,46
15-44	66,739	7708	769 (10.0%)	184 (24%)	0,24
45-64	31,156	3645	267 (7.3%)	62 (23%)	0,23
>64	18,640	1761	104 (5.9%)	21 (20%)	0,20

[1] Cumulative over the five seasons studied.

Table 13. Estimated incidences[1)] of influenza per 10,000 inhabitants of the specified age group in the general population of the Netherlands arranged according to season

Age group (yrs)	1992/93 B[2)]	1993/94 A/H3	1994/95 B+A/H3	1995/96 A/H3+B	1996/97 A/H3+B	mean
0-4	216	251	113	185	138	181
5-14	819	634	276	504	392	526
15-44	282	244	199	232	208	233
45-64	217	275	177	245	213	226
>64	163	198	102	168	164	159
All ages	312	306	199	271	271	272

[1)] The incidences were estimated by correcting the ILI incidences given in Table 6 with the laboratory-based correction factor calculated in Table 7, for the fraction of ILI patients who consulted their GP, and for the 5 day registration period per week. See section 3.6.1.
[2)] The dominant influenza virus (sub)types are presented for each season, in each case accounting for at least 85% of the total number of isolates obtained during the NIVEL/RIVM surveillance. When two (sub)types are given, the first was the most prevalent one.

3.6.3 Incidence of influenza: dependency on influenza virus (sub)type and age

As with age, the influenza correction factor differed for the three influenza virus (sub)types. The highest impact of influenza was found in 5-14-year-old children for influenza virus (sub)types A(H3N2) and B, namely 2.6% and 2.4%, respectively (Table 14). These (sub)types also had the greatest impact when averaged over all age groups. The A(H1N1) virus was by far the least prevalent of the three influenza viruses.

3.6.4 Incidence of influenza: dependency on degree of urbanization

The incidence of ILI was about the same in weakly and highly urbanized areas (Table 15). Unexpectedly, the lowest incidence was consistently observed in intermediately urbanized areas (towns). Taking the various influenza correction factors (Table 16) into account, the ratios of the three incidences changed considerably, but the estimated incidence of influenza in towns remained the lowest of the three (Table 17). The influenza incidence was highest in low-urbanized areas, ironically implying that influenza strikes hardest at the "healthy" countryside.The described peculiar ranking order was observed in each of the five studied seasons, ruling out mere chance as an explanation. The nature of the factors involved is obscure. One could think of artefacts

like a consistently different tendency of patients to consult their GP, or of GPs to register an illness as an ILI. Alternatively, the ranking order could be real and the causes of the peculiar ranking order should be sought in differences in the general condition of people or in their contacts with other individuals.

Tabel 14. Estimated incidences[1)] of influenza per 10,000 inhabitants of the specified age group in the general population of the Netherlands averaged over the seasons 1992/93 up to and including 1996/97 arranged according to virus (sub)type

Age group (yrs)	A(H3N2)	A(H1N1)	B	Total
0-4	125	19	37	181
5-14	260	28	238	526
15-44	113	11	109	233
45-64	138	6	82	226
>64	100	6	53	159
All ages	144	13	115	272

[1)] The incidences were estimated by correcting the ILI incidences given in Table 6 with the laboratory-based correction factor calculated in Table 7, for the fraction of ILI patients who consulted their GP, and for the 5 day registration period per week. See section 3.6.1.

Table 15. Incidence of ILI per 10,000 inhabitants registered by NIVEL calculated from week 40 up to week 20, arranged according to degree of urbanization and season

Degree of urbanization	1992/93	1993/94	1994/95	1995/96	1996/97	Mean of all seasons
Village	299	310	236	328	279	290
Town	235	240	153	219	184	206
City	400	357	226	283	281	309
Total	286	280	182	248	248	249

Table 16. Laboratory-proven influenza incidences in the 1992/93 up to and including 1996/97 seasons arranged according to urbanization

Degree of Urbanization	Mean patient population NIVEL	Number of ILI reported to NIVEL	Number of samples from ILI (%)	Number of flu-positive samples (%)	Lab-based correction factor
Village	20,703	2999	115 (4%)	41 (36%)	0.36
Town	88,915	9093	590 (6%)	141 (24%)	0.24
City	31,851	4868	689 (14%)	179 (26%)	0.26

Table 17. Estimated incidences[1)] of influenza per 10,000 inhabitants in the general population of the Netherlands arranged according to degree of urbanization and season

Degree of urbanization	1992/93 B[2)]	1993/94 A/H3	1994/95 B+A/H3	1995/96 A/H3+B	1996/97 A/H3+B	mean
Village	452	469	357	496	422	438
Town	237	242	154	221	185	208
City	437	390	247	309	307	337
Total	312	306	199	271	271	272

[1)] The incidences were estimated by correcting the ILI incidences given in Table 6 with the laboratory-based correction factor calculated in Table 7, for the fraction of ILI patients who consulted their GP, and for the 5 day registration period per week. See section 3.6.1.
[2)] The dominant influenza virus (sub)types are presented for each season, in each case accounting for at least 85% of the total number of isolates obtained during the NIVEL/RIVM surveillance. When two (sub)types are given, the first was the most prevalent one.

3.6.5 Incidence of influenza: dependency on region

Even in small countries like the Netherlands, influenza epidemics do not strike evenly. The main findings in the analysis of the regional spread of ILI were that the eastern provincies experienced the highest incidence, while the northern three provincies were relatively spared (Table 18). Here, the incidence of ILI was only half that in the eastern region. Introduction of the influenza correction factors (for their derivation see Table 19) does not change the picture much. The most affected region remains the east, while the three more northern provinces are still the least affected (Table 20). This was true for all five seasons studied. This phenomenon suggests that the observed ranking order of the incidence of influenza by region is only weakly influenced by the specific immunity acquired during previous influenza epidemics and is mainly dependent on intrinsic geographical factors, whatever these may be. We could not detect any correlation between the distribution of influenza incidence by region and that by degree of urbanization.

Table 18. Incidence of ILI per 10,000 inhabitants registered by NIVEL, calculated from week 40 up to week 20, arranged according to province group and season

Province group	1992/93	1993/94	1994/95	1995/96	1996/97	Mean of all seasons
North	201	192	91	176	115	157
East	382	363	237	322	259	313
West	271	292	151	226	230	234
South	295	242	244	265	215	252
Total	286	280	182	248	248	249

Table 19. Laboratory-proven influenza incidencesin the 1992/93 up to and including 1996/97 seasons arranged according to region

Province group	Mean patient population NIVEL	Number of ILI reported to NIVEL	Number of samples from ILI (%)	Number of influenza virus-positive samples (%)	Lab-based correction factor
North	29,023	1726	105 (6%)	24 (23%)	0.23
East	30,714	4741	278 (6%)	82 (29%)	0.29
West	54,630	6292	646 (10%)	183 (28%)	0.28
South	33,393	4193	365 (9%)	72 (20%)	0.20

Table 20. Estimated incidences[1)] of influenza per 10,000 inhabitants of province group in the general population of the Netherlands arranged according to province group and season

Province group	1992/93 B[2)]	1993/94 A(H3)	1994/95 B+A(H3)	1995/96 A(H3)+B	1996/97 A(H3)+B	Mean
north	194	185	88	170	111	152
east	465	442	289	392	315	381
west	319	343	178	266	270	275
south	248	203	205	223	181	212
Total	312	306	199	271	271	272

[1)] The incidences were estimated by correcting the ILI incidences given in Table 6 with the laboratory-based correction factor calculated in Table 7, for the fraction of ILI patients who consulted their GP, and for the 5 day registration period per week. See section 3.6.1.
[2)] For each season the dominant influenza virus (sub)types are presented, in each case accounting for at least 85% of the total number of isolates obtained from the NIVEL/RIVM surveillance. When two (sub)types are given, the first was the most prevalent one.

3.7 Age dependency of infections with all kinds of pathogens

During the 1992/93-1996/97 period, NIVEL-GPs took samples from about 8% of the patients presenting with ILI (Table 12). The age distribution of the sampled patients was well-matched to that of all patients with ILI: the percentage sampled varied from 6 to 10%. When examining the age distributions of the most prevalent pathogens, rhinoviruses and RS viruses showed a predilection for 0-4 year olds and influenza viruses for 5-14 year olds (Table 21).

Table 21. Detection of specified viruses in the period 1992/93 up to and including 1996/97 arranged according to age group1)

Age group (yrs)	Dutch population on 1-1-96 (%)2)	Samples examined (%)	influenza virus A(H3N2)	influenza virus A(H1N1)	influenza virus B	influenza virus total	rhinovirus	RS virus	coronavirus
0-4	981,000 (6%)	189 (8%)	20 (11%)	3 (2%)	6 (3%)	29 (15%)	38 (20%)	25 (13%)	0 (0%)
5-14	1,867.000 (12%)	235 (11%)	46 (20%)	5 (3%)	42 (18%)	93 (40%)	14 (6%)	3 (1%)	3 (1%)
15-44	7,015.000 (45%)	1198 (54%)	114 (10%)	11 (1%)	111 (9%)	236 (20%)	165 (14%)	16 (1%)	17 (1%)
45-64	3,570.000 (23%)	435 (19%)	47 (11%)	2 (1%)	28 (6%)	77 (18%)	56 (13%)	11 (3%)	10 (2%)
>64	2,061.000 (13%)	177 (8%)	17 (10%)	1 (1%)	9 (5%)	27 (15%)	17 (10%)	2 (1%)	3 (2%)
All ages	15,494.000 (100%)	2234 (100%)	244 (11%)	22 (1%)	196 (9%)	462 (21%)	290 (13%)	57 (3%)	33 (1%)

1) During the 1994/95 and 1996/97 seasons, the samples were tested for rhinovirus, enterovirus, RS virus, coronavirus OC43, coronavirus 229E, Mp, and Cp using PCR.
2) The data on the Dutch population were supplied by Statistics Netherlands (CBS).

pag.44 van 59 Rapport nr. 245607 005

3.8 Relation between clinical data and infection

3.8.1 Association between clinical symptoms and specified infections

We composed a table listing the symptoms reported by the NIVEL GPs for the patients examined in the virological NIVEL/RIVM surveillance of respiratory virus infections over the five studied seasons, arranged by pathogen (Table 22). As expected, the symptom profiles of the various infections largely overlapped each other. With respect to virus infections, *cough* was most prominent with infections of Mp, Cp, influenza virus, RS virus, and coronavirus; *rhinorrhoea* with RS virus and rhinovirus; *sore throat* with parainfluenza virus and coronavirus; *fever* or body temperature above 39^{0}C with influenza virus, adenovirus, RS virus, and enterovirus; *malaise* with adenovirus, coronavirus, and influenza virus; *myalgia* with influenza virus, parainfluenza virus, adenovirus, and enterovirus; *headache* with Cp and influenza virus; and *gastrointestinal complaints* with adenovirus, RS virus, and influenza virus.

Overlapping symptomatology was also found with infections with the various (sub)types of influenza virus (Table 23). Rhinorrhoea occurred more frequently with influenza H1N1, while a temperature of over 39^{0}C was seen less frequently with influenza B. The *ILI label* was relatively most frequently observed with H3N2 virus infections.

O nly for infections with influenza viruses, RS viruses, and rhinoviruses were the numbers large enough to obtain statistically significant results. Table 24 compares pairwise the symptom profiles of these infections using the various odds ratios; those above 3.0 or below 0.3 are in bold print and underlined. We preferred to present odds ratios rather than relative risks, because when calculating odds ratios, properties occurring in higher frequencies are given more weight than the rarer ones. We found that ILI, malaise, and myalgia were registered more frequently for influenza virus, while "ILI-negative" was cited more often for RS virus and for rhinovirus infection. Influenza virus and RS virus infections were associated more frequently with fever or body temperature above 39^{0}C than rhinovirus infections. Influenza A(H3N2) virus infections could not be clearly distinguished from B virus infections nor B from H1N1; however, the ILI label was given more often to H3N2 infections than to H1N1 infections.

A more advanced statistical analysis of the association between clinical symptoms and specified infections is under preparation.

Table 22. Clinical symptoms of the virologically examined patients in the period 1992/93 up to and including 1996/97 arranged according to pathogen

Symptoms	Numbers (%) of patients showing the listed symptoms													
	influenza virus	RS virus	rhinovirus	entero-virus	coronav. OC43	coro. 229E	adeno-virus	para-influenza	Chlamydia pneumon.	Mycopl. pneumon.	ILI-positive	ILI-negative	negative	total
	475 (21%)	58 (3%)	295 (13%)	36 (2%)	31 (1%)	3	33 (1%)	21 (1%)	12 (0.5%)	14 (0.6%)	1388	629	1287	2234
ILI-pos.	366 (77%)	35 (60%)	149 (51%)	20 (56%)	14 (45%)	2	23 (70%)	10 (48%)	8 (67%)	10 (71%)	1388 (100%)	0 (0%)	778 (60%)	1388
ILI-neg.	46 (10%)	16 (28%)	131 (44%)	12 (33%)	13 (42%)	1	6 (18%)	8 (38%)	3 (25%)	4 (29%)	0 (0%)	629 (100%)	390 (30%)	629
Cough	412 (87%)	50 (86%)	210 (72%)	24 (67%)	26 (84%)	3	20 (61%)	14 (67%)	11 (92%)	13 (93%)	1076 (78%)	361 (57%)	847 (66%)	1608
Rhinorrhoea	243 (51%)	38 (66%)	175 (60%)	13 (36%)	13 (42%)	2	15 (45%)	8 (38%)	4 (33%)	4 (29%)	715 (52%)	318 (51%)	645 (50%)	1152
Sore throat	302 (64%)	35 (60%)	207 (71%)	26 (72%)	24 (77%)	3	20 (61%)	19 (90%)	9 (67%)	7 (50%)	993 (72%)	435 69%)	955 (74%)	1589
Red throat	171 (36%)	18 (35%)	119 (41%)	9 (25%)	11 (35%)	2	12 (36%)	12 (57%)	2 (17%)	5 (36%)	588 (42%)	246 (39%)	553 (43%)	901
Dyspnoea	32 (7%)	9 (16%)	28 (10%)	3 (8%)	2 (6%)	0	3 (9%)	2 (10%)	2 (17%)	1 (7%)	139 (10%)	19 (3%)	100 (8%)	181
Fever	430 (91%)	50 (86%)	169 (58%)	28 (78%)	24 (77%)	2	31 (94%)	13 (62%)	10 (83%)	10 (71%)	1224 (88%)	304 (48%)	956 (74%)	1702
Temp. <39	147 (31%)	21 (36%)	84 (29%)	12 (33%)	14 (45%)	0	14 (42%)	8 (38%)	6 (50%)	5 (36%)	486 (35%)	166 (26%)	441 (34%)	609
Temp. ≥39	225 (47%)	23 (40%)	54 (19%)	14 (39%)	4 (13%)	2	13 (39%)	4 (19%)	3 (25%)	4 (29%)	548 (39%)	97 (15%)	386 (30%)	716
Malaise	334 (70%)	25 (43%)	149 (51%)	19 (53%)	22 (71%)	2	26 (79%)	11 (52%)	3 (25%)	6 (43%)	1026 (74%)	217 (34%)	785 (61%)	1373
Myalgia	311 (65%)	20 (34%)	117 (40%)	18 (50%)	13 (42%)	1	17 (52%)	12 (57%)	3 (25%)	6 (43%)	913 (66%)	190 (30%)	713 (55%)	1222
Headache	269 (57%)	18 (31%)	94 (32%)	14 (39%)	16 (52%)	2	15 (45%)	8 (38%)	7 (58%)	3 (21%)	733 (53%)	182 (29%)	587 (46%)	1034
Nausea	44 (9%)	3 (5%)	17 (6%)	0 (0%)	1 (3%)	0	7 (21%)	1 (5%)	2 (17%)	1 (7%)	160 (12%)	33 (5%)	121 (9%)	194
Vomit	31 (7%)	7 (12%)	11 (4%)	3 (8%)	2 (6%)	0	2 (6%)	0 (0%)	1 (8%)	1 (7%)	99 (7%)	21 (3%)	80 (6%)	143
Diarrhoea	16 (3%)	3 (5%)	9 (3%)	1 (3%)	0 (0%)	0	4 (12%)	1 (5%)	0 (0%)	0 (0%)	57 (4%)	16 (3%)	45 (3%)	74

pag.46 van 59 Rapport nr. 245607 003

Table 23. Clinical symptoms associated with influenza virus infections arranged according to (sub)type in the 1992/93 - 1996/97 period

	Numbers (%) of patients showing the listed symptoms			
Symptoms	A(H3N2)	A(H1N1)	B	total influenza
	n = 251	n = 28	n = 196	n = 475
ILI-pos.	213 (85%)	15 (54%)	138 (70%)	366 (77%)
ILI-neg.	18 (7%)	5 (18%)	23 (12%)	46 (10%)
Acute start	233 (93%)	28 (100%)	181 (92%)	442 (93%)
Cough	223 (89%)	25 (89%)	164 (84%)	412 (87%)
Rhinorrhoea	127 (51%)	20 (71%)	96 (49%)	243 (51%)
Sore throat	165 (66%)	20 (71%)	117 (60%)	302 (64%)
Red throat	88 (35%)	10 (36%)	73 (37%)	171 (36%)
Dyspnoea	18 (7%)	0 (0%)	14 (7%)	32 (67%)
Fever	230 (92%)	26 (93%)	174 (89%)	430 (91%)
Temp. <39	68 (27%)	9 (32%)	70 (36%)	147 (31%)
Temp. ≥39	129 (51%)	16 (57%)	80 (41%)	225 (47%)
Malaise	181 (72%)	21 (75%)	132 (67%)	334 (70%)
Myalgia	163 (65%)	19 (68%)	129 (66%)	311 (65%)
Headache	136 (54%)	18 (64%)	115 (59%)	269 (57%)
Nausea	16 (6%)	3 (11%)	25 (13%)	44 (9%)
Vomit	19 (8%)	1 (4%)	11 (6%)	31 (7%)
Diarrhoea	8 (3%)	2 (7%)	6 (3%)	16 (3%)

Table 24. Odds ratios for clinical symptoms for the 1992/93 - 1996/97 period

Symptoms	influenza virus cp* with RS virus	influenza virus cp with rhinovirus	RS virus cp with rhinovirus	influenza A(H3N2) virus cp with influenza B virus	influenza A(H3N2) virus cp with influenza A(H1N1) virus	influenza B virus cp with influenza A(H1N1) viru
ILI-positive	2.2 (3)	**3.3 (1)**	1.5 (5)	2.4 (3)	**4.9 (1)**	2.1 (5)
ILI-negative	**0.3 (1)**	**0.1 (1)**	0.5 (4)	0.6 (5)	0.4 (5)	0.6 (5)
Cough	1.0 (5)	2.6 (1)	2.5 (4)	1.6 (5)	1.0 (5)	0.6 (5)
Rhinorrhoea	0.6 (4)	0.7 (4)	1.3 (5)	1.1 (5)	0.4 (4)	0.4 (4)
Sore throat	1.1 (5)	0.7 (5)	0.6 (5)	1.3 (5)	0.8 (5)	0.6 (5)
Dyspnoea	0.4 (4)	0.7 (5)	1.8 (5)	1.0 (5)	n.a.	n.a.
Fever	1.5 (5)	**7.3 (1)**	**4.8 (1)**	1.4 (5)	0.8 (5)	0.6 (5)
Temperature <39	0.8 (5)	1.2 (5)	1.5 (5)	0.7 (5)	0.8 (5)	1.2 (5)
Temperature ≥39	1.4 (5)	**4.5 (1)**	**3.3 (2)**	1.5 (5)	0.8 (5)	0.5 (5)
Malaise	**3.1 (1)**	2.3 (1)	0.7 (5)	1.3 (5)	0.9 (5)	0.7 (5)
Myalgia	**3.6 (1)**	2.8 (1)	0.8 (5)	1.0 (5)	0.9 (5)	0.9 (5)
Headache	2.9 (2)	2.7 (1)	0.9 (5)	0.8 (5)	0.7 (5)	0.8 (5)

* cp: compared

In parentheses:
- (1): $P \leq 0.0001$
- (2): $0.0001 < P \leq 0.001$
- (3): $0.001 < P \leq 0.01$
- (4): $0.01 < P \leq 0.05$
- (5): $P > 0.05$

Odds ratios above 3.0 or below 0.3 are in bold print and underlined.

3.8.2 Association between ILI and specified infections

As stated above, one of the reasons for the NIVEL/RIVM GP surveillance of respiratory viral illnesses was to investigate the various infectious causes of ILI as registered by the NIVEL GPs. This issue was addressed by composing "virus profiles" for "ILI-positive" and "ILI-negative patients" (Table 25). An influenza virus was detected in 26% of the ILI-positive patients, a rhinovirus in 11%, another virus/Mp/Cp in 9%, and no virus/Mp/Cp in 56% (column 2 of Table 25). In other words, the clinical diagnosis "influenza" was confirmed in the laboratory for 26% of the ILI-positive patients. In contrast, an influenza virus was detected in only 7% of the ILI-negative patients, a rhinovirus in 21%, another virus/Mp/Cp in 10%, and no virus/Mp/Cp in 62% (column 3 of Table 25). As expected, rhinovirus was found to be more prevalent among the ILI-negative patients. The ranking order of the contributions of H3N2, B, and H1N1 virus infections to the ILI syndrome is also apparent in this comparison: the diseases of H3N2, B, and H1N1 patients were labeled as ILI in 84%, 70%, and 54% of the cases, respectively (column 4 of Table 25).

3.8.3 Association between clinical diagnoses and virus infections

GPs participating in the virological NIVEL/RIVM surveillance of respiratory virus infections were asked to register not only the symptoms of the sampled patients but also their diagnosis. This enabled us to relate the diagnosis with the kind of pathogen isolated in the laboratory. Because of the low numbers of the various diagnoses, the pathogens included in this analysis were limited to influenza virus, rhinovirus, "other pathogen", and "no pathogen". Influenza virus was relatively most prevalent with otitis and pneumonia, rhinovirus with sinusitis, tonsillitis, and laryngitis (Table 26).

Table 25. Virus profiles for ILI positive and ILI negative patients for the 1992/93 - 1996/97 period

Respiratory pathogen	ILI-positive	ILI-negative	ILI+/ILI-	unknown	total
influenza A(H3N2) virus	213 (15.3%)	18 (2.9%)	5	20	251
influenza A(H1N1) virus	15 (1.1%)	5 (0.8%)	1	8	28
influenza B virus	138 (9.9%)	23 (3.7%)	3	35	196
total influenza virus	366 (26.4%)	46 (7.3%)	4	63	475
parainfluenza virus	10 (0.7%)	8 (1.3%)	0.5	3	21
rhinovirus	149 (10.7%)	131 (20.8%)	0.5	15	295
RS virus	35 (2.5%)	16 (2.5%)	1	7	58
adenovirus	23 (1.7%)	6 (1.0%)	2	4	33
enterovirus	20 (1.4%)	12 (1.9%)	0.7	4	36
coronavirus	16 (1.2%)	14 (2.2)	0.5	4	34
Mycoplasma pneumoniae	10 (0.7%)	4 (0.6%)	1	0	14
Chlamydia pneumoniae	8 (0.6%)	3 (0.5%)	1	1	12
no virus/Mp/Cp	778 (56%)	390 (62%)	0.9	119	1287
Total	1388	629	1	199	2216

Table 26. Virus/Mp/Cp detections arranged according to clinical diagnosis other than ILI (but often in combination with ILI) in the 1992/93 - 1996/97 period

Diagnosis	**no of samples**	**influenza virus**	**rhinovirus**	**other path.**	**no pathogen** *
sinusitis	157	20 (13%)	35 (22%)	12 (8%)	90 (57%)
otitis	50	6 (12%)	3 (6%)	6 (12%)	35 (70%)
conjunctivitis	26	4 (15%)	3 (12%)	5 (19%)	15 (58%)
pharyngitis	352	56 (16%)	52 (15%)	30 (9%)	214 (61%)
tonsilitis	74	3 (4%)	10 (14%)	7 (9%)	54 (73%)
laryngitis	97	9 (9%)	25 (26%)	14 (14%)	49 (51%)
bronchitis	127	21 (17%)	24 (19%)	9 (7%)	73 (57%)
bronchiolitis	17	2 (12%)	2 (12%)	2 (12%)	11 (65%)
pneumonia	30	5 (17%)	3 (10%)	3 (10%)	19 (63%)
all patients	*2225*	*475 (21%)*	*221 (10%)*	*208 (9%)*	*1363 (61%)*

* Due to double infections, percentages may add up to more than 100%.

3.8.4 Association between specific patient conditions and virus infections

Since the start of the NIVEL/RIVM surveillance of respiratory virus infections, GPs were also requested to register whether similar illnesses occurred in the patient's neighbourhood and whether she or he had received an influenza vaccination. In the 1995/96 and 1996/97 seasons the patient was also asked whether she or he suffered from respiratory allergy and whether she or he had regular contacts with children below the age of five years. Again, because of the low numbers, the various conditions were compared pairwise with respect to the total percentage of samples in which any pathogen was demonstrated.

The relationship between **allergy** and infection is complex. On the one hand, allergic attacks triggered by an allergen may mimic respiratory infections, a factor that would lower the rate of virus isolations. On the other hand, infections that would otherwise go unnoticed may precipitate allergic episodes, a phenomenon that may increase the chance of virus isolations from allergic patients. The available data show no difference between the positivity rate of allergic and non-allergic patients (Table 27). It is not clear whether the two factors mentioned above compensate one another or whether both are too small to be demonstrable.

Table 27. Association between detection of virus infections and allergy, contacts with 0 - 4 - year - old children, and similar infections in the neighbourhood

Condition	period	number	any pathogen
all patients	95/96-96/97	1023	466 (46%) *
allergy yes	95/96-96/97	50	24 (48%)
allergy no	95/96-96/97	826	386 (47%)
contact children yes	95/96-96/97	345	184 (53%) **
contact children no	95/96-96/97	530	228 (43%) **
all patients	92/93-96/97	2216	861 (39%)
similar infections yes	92/93-96/97	873	384 (44%)
similar infections no	92/93-96/97	1316	537 (41%)

* percentages of positive samples are calculated with respect to the number of samples entered in the previous cell.

** Difference is significant using a chi-square test, $P < 0.005$.

Because the rate of infections with all sorts of pathogens is highest among 0-4-year-old children (Table 6), **contacts with young children** suffering from respiratory illness are expected to show a greater chance of scoring positive on virological examination. Analyzed for the 1995/96 and 1996/97 seasons, 53% of specimens from patients who met frequently young children were positive for any pathogen, whereas 43% of specimens from patients who denied such contacts had a pathogen. When tested using a chi-square test, this difference was significant, $P < 0.005$ (Table 27), confirming the expectation.

The figures of Table 27 also show that influenza virus was isolated less frequently from patients of the NIVEL/RIVM-surveillance who received an **influenza vaccination** than from the group of all patients (9.5 versus 6.1%). On the basis of these data, one could calculate a 36% protection rate for those receiving the vaccine. This percentage is in accordance with published observations (37) but should be considered with great caution. The data for the two groups of patients were not matched for age or other important determinants of influenza virus infection and subsequent disease. When matched, the figures were too small to be submitted to statistical analysis. Moreover, the study was conducted among general practice patients. Only if one assumes that, irrespective of their vaccination status, individuals have the same chance of visiting their GPs if they have a similar respiratory disease, may one extrapolate the protection rate to all those vaccinated.

The occurrence of **similar illnesses** in the patient's neighbourhood is suggestive of the circulation of an infectious agent. Therefore, patients reporting this are expected to score positive more frequently on virological examination than other patients. Such patients did indeed display an enhanced rate of isolating a pathogen but the increase was insignificantly small (Table 27).

CONCLUSIONS

1. The NIVEL/RIVM surveillance was able to detect and characterise waves of respiratory illness caused by either influenza viruses or non-influenza viruses. In September 1996, as in Septembers of previous years, such a wave was indicated by an enhanced proportion of samples being positive for any respiratory agent. This activity, which coincided with the beginning of a new school year, was mainly associated with rhinovirus infections.

2. In the 1996/97 season, a potential respiratory pathogenic agent was detected in 64% of the specimens using culture and/or PCR. Viruses were found in 53%, Mp and Cp in 2.4%, and conventional bacteria in 16% of the samples.

3. PCR detection and cultivation of conventional bacteria have proven useful to the NIVEL/RIVM surveillance.

4. Again in the 1996/97 season, the surveillance functioned well as an early warning and aetiological characterization system for influenza epidemics. The first influenza A(H3N2) virus of this surveillance was isolated in week 51 of 1996, when the ILI incidence had hardly increased and well before the ILI peak in week 4 of 1997.

5. Over the 1992/93 - 1996/97 seasons, influenza virus infections accounted for at least 26% of the ILI registered by NIVEL. Over the five seasons studied, an estimated 2.7% of the general community developed an ILI caused by an influenza virus infection per season.

6. According to the ILI registration, the highest incidence of ILI was among 0-4-year-old children in all five seasons. (Table 11). After correction for the influenza virus isolation rate and the fraction of ILI patients who consulted their GP, however, the highest incidence of influenza, 5.3%, was among the 5-14-year olds (Table 13).

7. Influenza occurred most frequently in the (according to popular belief "healthy") country-side (T17) and least frequently in the northern region of the Netherlands (T18).

8. The NIVEL/RIVM surveillance should be continued in the format of a case-control study.

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RESPIRATORY INFECTIONS IN GENERAL PRACTICES (GPs) IN THE 1996/97 SEASON

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Background and methods

We performed a laboratory-based surveillance among patients with acute respiratory illnesses, presenting to one of the GPs of the network of NIVEL. Sixty-five percent of the examined patients had an influenza-like illness. The age distribution of the patients matched well the age distribution of the Dutch population. The clinical samples were cultured for viruses and bacteria and tested by nested PCR for selected viruses and bacteria.

Results and Discussion

September.
In the Netherlands, the respiratory 1996/97 season started in September with the usual increase of the proportion of specimens in which a pathogen, in most cases a rhinovirus, was detected (*Figure 1*). This increase probably represents a real wave of respiratory illnesses (as is actually observed in the surrounding countries), coinciding with the opening of schools at the end of August.

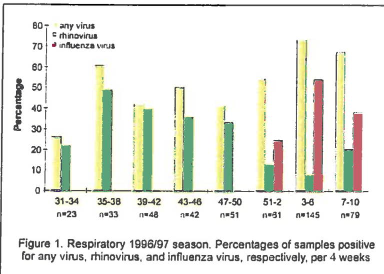

Figure 1. Respiratory 1996/97 season. Percentages of samples positive for any virus, rhinovirus, and influenza virus, respectively, per 4 weeks

Influenza.
The 1996/97 influenza epidemic was average with respect to time of appearance, duration and severity (*Figure 2*). As reported before (ECJ Claas et al., Lancet 1995; 346:180), the ratio of the number of isolates of subtype A(H3N2) to that of type B was considerably higher for the Dutch hospital-based diagnostic laboratories than in the GP study in the whole period 1992-1997 (*Figure 3*). This probably reflects the higher pathogenicity of subtype A(H3N2) compared with type B.

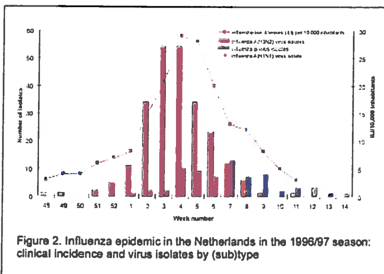

Figure 2. Influenza epidemic in the Netherlands in the 1996/97 season: clinical incidence and virus isolates by (sub)type

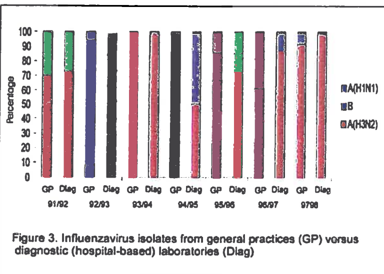

Figure 3. Influenzavirus isolates from general practices (GP) versus diagnostic (hospital-based) laboratories (Diag)

Other pathogens.
Calculated over the whole year, influenza virus, rhinovirus, and Streptococcus haemolyticus occurred most frequently (*Table 1, Figure 4*). In 64% of the samples one and in 10% two or three pathogens were detected. Compared with the results of the diagnostic laboratories, more influenza viruses and rhinoviruses, and less RS viruses were detected with the GP patients. These differences may be due to differences in age distribution, severity of the diseases, and diagnostic techniques

Table 1

Results virus/bacterium culture and -PCR of the NIVEL/RIVM GP surveillance of respiratory "viral" illnesses in the 1996/97 season

Respiratory pathogen	Positive: culture	PCR	follow-up samples
540 virological specimens: 15 July 1996 - 13 July 1997			
rhinovirus	22 (4.1%)	119 (22.0%)	
enterovirus	3 (0.6%)	21 (3.9%)	
RS virus	4 (0.7%)	29 (5.4%)	
influenza A(H3N2) virus	78 (14.4%)		
influenza B virus	49 (9.1%)		
parainfluenza virus	5 (0.9%)		
adenovirus	6 (1.1%)		
coronavirus OC43		9 (1.7%)	
coronavirus 229E		0	
Mycoplasma pneumoniae		7 (1.3%)	
Chlamydia pneumoniae		6 (1.1%)	
Culture-positive samples	**167 (30.9%)**		
PCR-positive samples	**191 (35.4%)**		**still PCR-positive$:**
Total culture- and/or PCR-positive	**296 (54.8%)#**		**1/25 (4%) (RS virus)**
Idem, corrected for long shedders	**284 (53.4%)**		

159 bacteriological samples: 5 November 1996 - 13 July 1997 (all 159 concerning patients were also virologically examined)		
Streptococcus haemolyticus A	7 (4.4%)	
Streptococcus haemolyticus B	3 (1.9%)	
Streptococcus haemolyticus C	1 (0.6%)	
Streptococcus haemolyticus F	1 (0.6%)	
Streptococcus haemolyticus G	1 (0.6%)	
Streptococcus pneumoniae	2 (1.3%)	
Haemophilus influenzae	5 (3.1%)	
Haemophilus parainfluenzae	3 (1.9%)	
Staphylococcus aureus	2 (1.3%)	
Enterobacter	2 (1.3%)	
Escherichia coli	1 (0.6%)	
Moraxella catarrhalis	0 (0.0%)	
Bacterial infections	**28**	**still bact. positive$:**
Bacterium-positive samples	**26 (16.4%)**	**1/8 (12%)@**
Bacterium-positive/virus-negative samples	**14 (8.8%)**	
Idem, corrected for long shedders	**12 (7.5%)**	

Total VIR - and/or BAC - positive samples: 63.6%
Total VIR - and/or BAC - positive samples, corrected for long shedders: 60.9%

$ After on an average 30 days (VIR) and six weeks (BAC) one recovered patient was still positive.
# In 1994/95 season: 35%
@ Streptococcus haemolyticus group A.

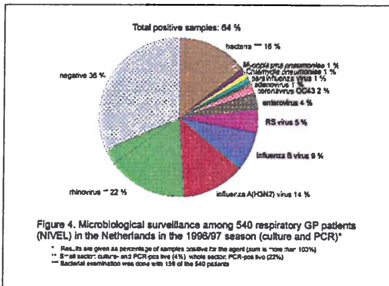

Figure 4. Microbiological surveillance among 540 respiratory GP patients (NIVEL) in the Netherlands in the 1996/97 season (culture and PCR)*

* Results are given as percentage of samples positive for the agent (sum is more than 100%)
** Small sector: culture- and PCR-positive (4%), whole sector: PCR-positive (22%)
*** Bacterial examination was done with 159 of the 540 patients

Duration of shedding.
On an average 31 days after the first sample, we took follow-up samples from 44 patients who had been RT-PCR-positive for rhinovirus or RS virus. When the patient at the time of the second sample still displayed respiratory symptoms, 7 of 19 patients were still positive by PCR for the same pathogen. Only one of the 25 recovered patients still tested positive by PCR. After correction for such prolonged virus shedders, in 61% of all clinical specimens one or more pathogens were detected (*Table 1*).

Conclusions

- Respiratory disease in patients of GPs seems mostly due to influenza virus, rhinovirus, and Streptococcus haemolyticus.
- The GP results differ considerably from those of diagnostic laboratories and can be expected to reflect more closely the proportions of pathogens prevalent in the general population.
- Illnesses from influenza B virus infections are generally less severe than those from influenza A(H3N2) virus infections.
- In recovering patients, positive PCR results, including those obtained with the sensitive rhino/enterovirus-PCR, are strongly correlated with respiratory disease.

Appendix 2.

Inzendformulier NIVEL/RIVM-project voor de virologische surveillance van influenza-achtige aandoeningen

Naam arts:

Naam patiënt:

Geboortedatum patiënt: Geslacht: M/V

Datum materiaal: Ziekteduur: dagen

Aard materiaal: neus keel (liefst beide!)

Symptomen:		**Eventueel de diagnose:**
Acuut begin		Sinusitis
Hoesten		Otitis
Rhinorrhoe		Conjunctivitis
Keelpijn		Pharyngitis
Rode keel		Pseudocroup
Dyspnoe		Tonsillitis
Koorts	.........°C	Laryngitis
Malaise		Bronchitis
Spierpijn		Bronchiolitis
Hoofdpijn		Pneumonie
Buikpijn		Tracheïtis
Misselijk		
Braken		
Diarree		Andere gegevens:

- Soortgelijke zieken in omgeving: ja/nee
- Gerapporteerd als IAZ: ja/nee
- Influenzavaccinatie voor dit seizoen: ja/nee
- Is de patiënt bekend met een respiratoire allergie?: ja/nee
- Heeft patiënt regelmatig contact met kind(eren) onder de 5 jaar?: ja/nee

N.B.: - De onbeënte transportvloeistof kan bij kamertemperatuur worden bewaard. De vloeistof blijft op deze wijze twee jaar bruikbaar.
- Na beënting het monster direct versturen. Kan dit niet, dan bewaren bij 4°C (niet invriezen).
- Materiaal afnemen tot uiterlijk 5 dagen na het begin der ziekte, in later afgenomen materiaal te weinig virus.
- Materiaal afnemen op maandag tot en met donderdag.

Niet invullen door inzender

Datum ontvangst materiaal:

Uitslag: Datum:

Contactpersoon: J.C. de Jong, tel.: 030 274 2284/2391 fax: 030 274 4449
Laboratorium : tel.: 030 274 2392/2247 fax: 030 274 4449

Appendix 3. ARI and ILI in Belgium 1995/96/97.

Ministerie van de Vlaamse Gemeenschap - Ministère de la Communauté Française

Surveillanceprogramma van ACUTE LUCHTWEGENINFECTIES en van GRIEP

Weekrapport toestand op : 16/3/1997 (week 11)

Aantal gevallen/100 patiëntencontacten

Bron : Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
Afdelingen Epidemiologie en Virologie
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IHE/GJ
IRA_9597

totale ALI

epidemische drempel

griepale ALI

40 35 30 25 20 15 10 5 0

S O N D J F M A M J J A S O N D J F M A

1995 1996 1997

De surveillance van acute respiratoire infecties en griep gaat door, maar de curve zal niet meer verschijnen tot september.

Om de monitoring te verbeteren, kunnen huisartsen die aan het surveillance netwerk willen deelnemen zich kenbaar maken maken via een gewoone brief, per fax of E-Mail te sturen op bovenvermeld adres.

Wij danken in het bijzonder de huisartsen die vorig seizoen hebben deelgenomen voor hun waardevolle gegevens.

week	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Influenza A						1		2	1	1			2	1	4	12	34	25
Influenza B																		
Influenza (totaal)						1		2	1	1			2	1	4	13	35	27
RSV	5	2		5	7	5	2	5	11	41	47	48	64	106	107	137	132	90
Parainfluenza	4	3	4	2	5	1	11	13	12	14	10	6	1	6	7	6	2	6
Adenovirus	6	3	5	4	12	7	4	10	7	13	8	10	6	7	9	11	10	6
Str. pneumoniae	7	11	18	21	26	29	20	19	27	18	24	18	18	30	28	32	43	44
Myc. pneumoniae	8	3	7	6	2	2	15	19	8	9	15	13	14	36	25	33	23	9
Chl. pneumoniae	5	6	3	4	5	5	3	7	4	4	4	7	2		1		4	2
Haem. influenzae	1	2	5	5	4	3	11	6		2	6	8	1	15	18	15	12	6

week	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
Influenza A	57	57	82	91	46	35	16	5	9	3							
Influenza B		2	1	9	10	10	8	8	15	3							
Influenza (totaal)	57	60	85	100	61	47	24	13	26	7							
RSV	112	118	76	67	74	53	38	43	30	42							
Parainfluenza	1	1	7	6	4	3	9	6	8	3							
Adenovirus	19	11	15	8	13	4	15	13	2	10							
Str. pneumoniae	52	22	49	47	49	34	46	24	12	14							
Myc. pneumoniae	26	37	25	44	22	15	24	16	25	5							
Chl. pneumoniae	4	8	2	4		1	1	2	4								
Haem. influenzae	9	11	13	18	20	13	16	14	9	5							