EPIDEMIOLOGY OF VIRAL RESPIRATORY TRACT INFECTIONS

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1.1 INTRODUCTION

Episodes of respiratory viral infection were recognized long before their causal agents were discovered, and viral epidemiology was one of the first branches of virology to be developed. Studies of respiratory viral epidemiology have been limited by the capabilities of the diagnostic methods available at the time. These have improved steadily over the years and are continuing to do so, particularly with the recent development of molecular methods of viral detection. Early studies tended to concentrate on those viruses that could be most easily detected using traditional methods, such as influenza viruses, adenoviruses and respiratory syncytial (RS) virus. As time passed rhinoviruses and coronaviruses were discovered, and as methods for their detection have improved, so has come an appreciation of their importance in respiratory viral illness. These two viruses are now thought to account for between 50% and 75% of upper respiratory tract infections, and as such, due emphasis will be given to these virus types in this chapter.

As our knowledge of the pathogenesis and molecular basis of respiratory virology has increased, a better understanding of the epidemiology of respiratory viral infection has resulted. In this chapter we will consider some general principles of respiratory virology, the biology of the common respiratory viral types and its influence on respiratory viral epidemi-

ology, and finally the epidemiology of the two most common respiratory viral syndromes: the common cold and acute exacerbations of wheezing illness.

The recent advance in molecular biology has led to the development of polymerase chain reaction (PCR)-based assays for the detection of most respiratory viral types, and use of these methods in carefully designed studies promises to increase even further our understanding of respiratory viral epidemiology. At the end of the chapter we discuss the results of some very recent studies using these new methodologies.

The epidemiology of bacterial infections of the respiratory tract, infections of the sinuses and ear, and respiratory infections associated with immunodeficiency are not discussed in this chapter.

1.2 EPIDEMIOLOGICAL DEFINITIONS

1.2.1 INCIDENCE AND PREVALENCE

Epidemiology quantifies disease occurrence within populations, and to achieve this, criteria need to be established to define rates of disease occurrence. The most commonly used measures are incidence and prevalence. Rates are fractions in which the numerator is the number of cases of disease and the denominator is a measure of the population.

Incidence (also called attack rate) is mainly used for acute diseases of short duration, or to define the number of new cases of a more chronic disease occurring in a certain period of time. A population and a time frame are defined, and the number of new cases occurring in that population during that interval of time is counted. The denominator includes both size of population and time frame. The incidence rate is then expressed as 'cases per million person-years' or a similar term. However, the time element is often omitted in expressing incidence, leaving the reader to determine the time frame from the context.

Prevalence is most frequently used for chronic diseases, particularly where onset is insidious and not readily dated. When point prevalence is calculated, a particular date is selected and the population recorded for that day constitutes the denominator; all new and previously existing cases identified on that date constitute the numerator. Prevalence is expressed simply as a rate with no time parameter. Frequently prevalence is expanded into a finite time, such as a month or year, in which case the rate is denoted as period prevalence.

1.2.2 STUDY DESIGNS

Prospective studies

Two groups are selected from a population under study, one with (index) and one without (control) a specified attribute. Both groups are followed prospectively for the incidence of the disease under study, and incidence rates for both groups are calculated. An important assumption governing the validity of such studies is that the two groups are at equal risk of the disease under study. For a variety of reasons, this assumption is often not fulfilled; differences between the two groups may then be minimized by dividing each according to parameters such as age, race, socioeconomic status, and then comparing rates for each subgroup.

Longitudinal studies

It is not always appropriate to a study design, or practically possible to select accurately matched index and control groups. Thus for some studies, a great deal of information about a particular disease can be obtained by studying a single group longitudinally. A great many studies of respiratory viral epidemiology have used such a study design, which has the important advantage that the outcome being studied is defined before the beginning of the study. In particular, many of the studies examining the epidemiology of the common

cold, and attempting to demonstrate an association between respiratory viral infections and exacerbations of asthma, have used such a study design.

Retrospective studies

Prospective studies are expensive and time consuming because they require the enrolment of large numbers of subjects who must be followed for a period of months or years: the less frequent the expectation of outcome under study, the larger the population and the longer the follow-up that will be needed. An alternative approach is to carry out retrospective case-control studies. These necessarily have a great potential for the introduction of bias in the selection of the groups to be studied, as the outcome is already known. However, they are frequently used for initial studies to attempt to identify associations, with prospective studies then being justified to confirm or refute the findings of retrospective studies. It is of great importance that the two samples (cases and controls) are representative of the populations from which they are drawn, as the main flaw in retrospective studies is the choice of cases and particularly the selection of representative controls.

1.3 FACTORS INFLUENCING RESPIRATORY VIRAL EPIDEMIOLOGY

1.3.1 VIRAL DIAGNOSIS

Diagnosis of respiratory viral infections has classically been based on detection of the virus in nasal secretions, or on detection of an immune response in the blood (see Chapter 4). Nasal secretions (either washings or an aspirate) are the best specimens for viral detection, with maximal isolation rates being achieved within 1 or 2 days of the onset of symptoms. Specimens are inoculated onto a variety of cultured cell lines and viral presence detected by typical cytopathic effects, or by other testing such as haemagglutination. In addition nasal epithelial cells can be cytospun onto microscope slides, and virus detected by fluorescence with specific antibodies. Detection of a humoral immune response initially used tests such as complement fixation, but in general these have now been superseded by more sensitive and specific tests such as enzyme-linked immunosorbent assays (ELISAs).

These classical methods are relatively successful for the diagnosis of respiratory viral infections such as influenza, parainfluenza, RS virus and adenovirus, though even with these, great care in the handling and processing of specimens is required. However, rhinoviruses and coronaviruses (which together account for between 50–75% of acute respiratory viral infections), do not grow on standard cell lines, and antibodies are not detected easily. These viruses are therefore frequently not sought in epidemiological studies, and even if they are, it is generally acknowledged that the methods used are suboptimal. Even with the most exhaustive testing including the use of organ cultures and passage in human volunteers, under-diagnosis is likely, with infectious agents being demonstrable in only 60% of common colds [146].

Recent advances in molecular biology have permitted the sequencing of entire genomes of representative serotypes of almost all of the common respiratory viruses. This knowledge has permitted the development of PCR assays to provide sensitive and specific diagnosis. Such assays are under development for all the respiratory viruses, and in some cases have been used in epidemiological studies. Particularly for rhinoviruses (where PCR has been shown to be between three and five times more sensitive than culture) and coronaviruses, this has allowed much greater insight into the true role played by these viruses in acute respiratory illnesses [125,126,179]. The results of previous epidemiological studies should therefore be interpreted in the light of this knowledge, and it should be particularly borne in mind that

rhinoviruses and coronaviruses are likely to be underrepresented in figures quoted.

1.3.2 IMMUNITY

Most acute viral infections confer life-long immunity, mediated by a combination of local and systemic antibody responses, and cell-mediated immunity. Re-exposure at any interval after initial infection then results in (a) a re-infection with minimal virus replication, or (b) an anamnestic immune response. Such re-infections are frequently covert, and result in minimal shedding of infectious virus. For certain viruses, such as poliovirus or rhinovirus, immunity is type-specific and confers little protection against exposure to a different serotype. These facts have implications for respiratory viral epidemiology, as a population may therefore be divided into susceptible and immune subjects. Immune subjects are largely exempt from disease and are inefficient links in a transmission chain, whereas susceptible subjects can both spread the agent and experience disease. There are, however, well-proven exceptions to this pattern. Thus, most children will be infected with RS virus within the first year of life, but re-infection later in childhood and indeed in adulthood is more the rule than the exception. Similarly, re-infections occur with influenza virus, as a result of antigenic drift, and antigenic shift (see below), and it is likely that they also occur with the human strains of coronavirus, and several of the other respiratory viruses as well.

1.3.3 SEASONALITY

Many acute viral infections exhibit striking seasonal patterns in incidence which are very consistent. These patterns reflect seasonal differences in transmission of infection. Respiratory infections spread more readily in the winter, although they may peak at different times, and these times may vary from one year to another.

The underlying biological explanation for seasonal differences has remained elusive. However, it is clear that these variations correlate with changes in climate, including changes in humidity, barometric pressure, winds, precipitation and drought. These factors can affect infectious disease agents directly. Many viruses in the free state are vulnerable to heat, radiation and drying.

Overall, respiratory infections are more frequent in the colder months but within this period there are considerable variations in the relative prevalences of the individual viruses. Rhinoviruses, for example, peak in early and late winter, while influenza viruses are most active in midwinter, and at least in the UK, RS virus has a well-defined peak in mid–late winter. Both older and more recent studies have highlighted the strength of the relationship between viral respiratory infections and school term times, suggesting that for rhinoviruses and coronaviruses, the most important factor controlling the seasonal variability in incidence is the increased congregation of pre-school and school age children indoors facilitating airborne transmission [27,125,180] (Figure 1.1). For other viruses such as influenza or RS virus, with more clearly demarcated mini-epidemics, it is likely that factors such as fluctuations in temperature and humidity are also important.

1.3.4 SOCIOECONOMIC FACTORS

The influence of socioeconomic factors depends to varying degrees on the density and distribution of populations, the level of social, political, cultural and scientific development and, most importantly, the inter-relations of people. Socioeconomic factors typically affect health by indirect means, and because they are often closely inter-related, the impact of individual factors is very difficult to assess. The relation of population density to the occurrence of infectious disease is substantial. Increasing density favours the spread of

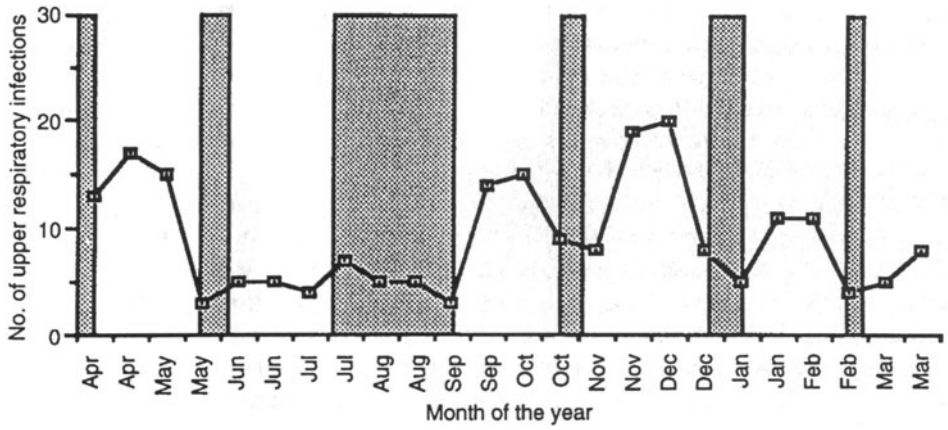

Figure 1.1 The seasonal variation in upper respiratory tract viral infections and its relationship to school attendance. Half-monthly incidence of viral infections in a cohort of 108, 9 to 11-year-old school children in Southampton, UK for the year April 1989–1990. School holiday periods are marked in shaded areas. (Adapted from [125].)

infectious agents, the occurrence of related disease and the development of immunity. In large and dense populations, agents typically infect early in childhood and persist because sufficient new susceptible subjects are added continuously by birth. In smaller populations the agents are unable to persist and are re-introduced at unpredictable intervals so that childhood diseases may be long delayed. Populations of urban and rural areas differ not only in relative density but also in other important ways, such as social structure, nutrition, air pollution, smoking, housing and family structure. The influence of many of these factors on respiratory viral infections has not been studied in detail, but several studies have addressed the important influence of family structure (see below).

The basic population unit is the household. Family members are genetically similar, share a common diet and economic status, are subject to the same cultural, religious and educational influences, and are exposed to common physical and biological environments. Most important for contact-transmitted disease, intra-familial contacts are prolonged and increase in intimacy with household crowding. Family size, regardless of degree of crowding, is particularly important for acute respiratory infections, since it determines the number of potential introducers who bring home infections acquired elsewhere.

1.3.5 FAMILY STRUCTURE

Much of our current knowledge on the epidemiology of respiratory viral infections comes from studies involving the continuing observation of family units for episodes of infection. These studies, the Virus Watch programs in New York and Seattle, monitored family members for specific infections revealed by virus isolation and/or antibody response, whether related to illness or not [31,32,51,53,56–63,91,98,137,142,211]. The family studies began with one member's infection, acquired from outside the house. This member then exposed his or her fellow family members. The introductory infection and any infections in those exposed constituted a family episode that was described in terms of the time of onset of the related infections and the identities (age, sex, position in the family) of the introducer and both the infected and uninfected contacts. School-age and pre-school age

children were identified as the most frequent introducers (hence important in community spread). This is consistent with the observed relationship between the crowding of children at school and the spread of respiratory viral infections, as these same children acquiring infections at school will then act as the introducers to the family unit.

Analysis also yielded estimates of cross-infection risks within the family, expressed in terms of secondary attack rates among specified members (for example, younger children) exposed to specified introducers (for example, a school child or a parent). In general, risk of contacts was related inversely by age overall, reflecting the influence of immunity, and of intimacy of within-family contact (ready exchange between spouses and between children nearest in age). Finally, the time relation between onsets of illness in the introducer and those exposed was used to define the range of incubation periods.

Studies of the Virus Watch type made it possible to identify and analyse family episodes caused by specific viruses (influenza A) or groups of viruses (adeno- or rhinoviruses) including both subclinical and overt infections. Analysis of the episodes also yielded information concerning the mode and duration of viral shedding; the spectrum of clinical response to infection, including the proportion that was subclinical; and the significance of prior immunity in the face of close exposure, as measured by the frequency and clinical consequences of re-infections that result.

1.4 SPECIFIC VIRUSES

There are in the region of 300 viruses or atypical bacteria that are known to be associated with respiratory tract infections. The more common of these will be considered individually, and aspects of their biology that relate to their epidemiology will be discussed. Details on those that have not been mentioned specifically in this chapter can be found within their own relevant chapters elsewhere in this text. The most common agents causing respiratory tract disease are listed in Table 1.1.

1.4.1 RHINOVIRUSES

Virus types and characteristics

Over 115 different serotypes of human rhinoviruses have been identified on the basis of serum neutralization studies [34,100,133,158]. It is likely, however, that new serotypes are constantly emerging under pressure from immune surveillance. Although most of the human rhinoviruses are antigenically distinct,

Table 1.1 The common infectious agents causing acute respiratory tract disease

Common viruses that cause acute respiratory illness	Rhinoviruses Coronaviruses 229E and OC43 Parainfluenza viruses types 1–4 Influenza viruses A, B and C Respiratory syncytial virus types A and B
Common infectious agents that may cause acute respiratory illness	Adenoviruses Enteroviruses Cytomegalovirus Herpes simplex virus *Mycoplasma pneumoniae* *Chlamydia pneumoniae* *Chlamydia psittaci*

cross-relationships between them have been detected [33]. Recent studies have revealed that 90% of rhinoviruses (major group) use the intercellular adhesion molecule-1 (ICAM-1) as their cellular receptor, while all but one of the remaining rhinoviruses (minor group) use a separate, and as yet only partially characterized receptor [223]. The minor group receptor has now been identified as the low density lipoprotein (LDL) receptor. All the rhinoviruses so far identified are capable of causing the common cold, and appear to cause a similar spectrum and severity of clinical illness.

Mode of transmission

Rhinovirus infections are spread from person to person by means of virus-contaminated respiratory secretions. Rhinoviruses are present in particularly high concentrations in nasal secretions rather than in pharyngeal secretions or saliva [86,109]. In studies of natural infections, higher rates of transmission were observed with longer exposures [41,57,86], and as discussed in previous sections, close contact (e.g. among family members) and crowding (e.g. school children) increase the transmission of rhinoviruses [42,115]. Early workers failed to demonstrate significant airborne transmission [37,89]. A more recent report, however, confirmed that rhinoviruses are transmitted by aerosol: in an experimental setting in which finger and fomite transmissions were prevented mechanically, transmission continued at the same rate [47], while the use of virucidal tissues in a family setting had no significant effect on rates of spread of colds [52]. These data, and the wealth of epidemiological data certainly support airborne transmission as the more important method. However, it is likely that both airborne and direct contact routes (contaminating hands, fingers and fomites with direct inoculation of virus through the conjunctiva and/or nasal epithelium) play a role in the transmission of rhinoviruses, as volunteer experiments show that saliva, lips and the external nares of infected volunteers contain small concentrations of virus [41], sufficient to initiate an infection in an individual provided that the virus successfully reaches the appropriate portal of entry [86,221].

Clinical course of rhinoviral infection

Clinical infection in the vast majority of cases is characterized by the typical symptoms of a cold which are indistinguishable from those caused by other viruses. The common cold is characterized by rhinorrhoea, nasal obstruction, pharyngitis and cough. Fever and systemic illness are infrequent though they may occur. The length of illness is normally around 7 days, with a peak of symptoms and signs between the second and third days. The acute phase of rhinitis usually corresponds with peak virus excretion [50]. Symptoms usually subside by the eighth day, although in some patients symptoms may persist for up to 1 month [39,86]. Lower respiratory tract symptoms are frequently present during common colds, especially cough which was present in 86% [54], 71% [175] and 40% [198] of normal subjects with naturally occurring infection.

There is also increasing evidence that rhinoviruses may be implicated in more severe lower respiratory tract illness, such as bronchiolitis, pneumonia and exacerbations of asthma, and indeed that they may directly infect the lower respiratory tract. A large number of epidemiological studies have strongly implicated rhinoviruses in exacerbations of asthma and as a cause of 'wheezy bronchitis' in children [126,191], and experimental rhinoviral infection studies have demonstrated the development of bronchial hyperreactivity during rhinoviral infection [8], as well as the development of late asthmatic responses in a group of atopic subjects [150].

Horn *et al.* have provided evidence for lower airway rhinoviral infection in that sputum cultures from 22 children with wheezy bronchitis were more often positive than either nose or throat swabs, suggesting that viral replication had occurred in the lower airways [118]. Cytopathic effects (CPE) developed more quickly in cultures from expectorated sputum as compared with upper airway samples implying that more virus was present in the lower airways.

Post-mortem studies have demonstrated that rhinoviruses are able to infect lung tissue in patients with compromised immunity. This has been seen in a patient with myelomatosis in whom RV13 was recovered from three specimens of lung tissue [39], and rhinovirus 47 was cultured post mortem from an 11-month-old infant with a history suggestive of bronchial asthma who had died suddenly at night [147]. Similar severe lower respiratory tract illnesses have been ascribed to rhinoviral infection in recent case reports [202].

Further evidence for a role for rhinoviruses in severe lower respiratory tract illnesses comes from three recent studies in New Zealand, Europe and the USA. In the first rhinoviruses were isolated from 20 children with bronchiolitis, and from 12 children with pneumonia over a 3-year period [155]. It was concluded that rhinoviral infections are less frequent than RS virus infections in infants (0.7% versus 8.2% of hospital admissions), but that the severity of illness and clinical presentations were similar. In the second study, rhinovirus was also identified as a cause of severe lower respiratory tract illness, including cough, wheeze, fever, respiratory distress and feeding difficulties; 70% of cases also had new chest X-ray abnormalities [136]. In the third study, rhinoviruses were detected in 12% of children requiring hospitalization for acute respiratory diseases including bronchiolitis and pneumonia; in comparison, RS virus was detected in 23%, but there were no differences between the clinical diseases caused by the two virus types [143]. It should be noted that these studies may well have underestimated the importance of the contributions of rhinoviruses as they employed cell culture only; the use of PCR would probably have increased rhinovirus detection considerably.

Prevalence of rhinovirus infections, age and sex distributions

The large number of different serotypes of rhinovirus together with the fact that infection with one serotype does not confer immunity to another, dictates that rhinovirus infections are very common. It has been estimated from studies using standard methods of detection in the USA that on average the incidence of rhinovirus infection is about 0.5 per person per year [86]. The mean rate for Seattle families was 0.68 infections per person-year [57], and rates in Virginia were higher [87]. Adjustment of the Seattle rates to reflect the Virginia rates indicates that infants and small children average more than one rhinovirus infection per year. Rates for young adult females in Virginia and in a Tecumseh, Michigan survey were higher than those for males of similar age [87,171]. A greater exposure to young children is thought to account for this difference.

All these studies relied upon viral culture to detect rhinovirus infection, and are therefore likely to have considerably underestimated the true prevalence of infection. A recent study using PCR in 9 to 11-year-old children in the UK found infection rates of 1.5 per child per year [126]. True rates of infection are probably even higher, since even with the use of PCR, the detection of rhinovirus infection is likely to be less than 100% as a result of incomplete reporting of symptoms, so a true estimate of the rate of infection is very difficult to obtain. The same UK study has revealed a great deal of unreported symptomatology, as well as an appreciable asymptomatic infection point prevalence of 12% [126]. Indeed, illness

induced by rhinoviruses probably represents the most common acute infectious illness of humans.

Infection is most common in early life, and incidence declines with increase in age, probably because of a combination of environmental factors such as reduced inter-personal contact, and the higher frequency of immunity among adults who have experienced infection by a larger number of rhinoviruses. Indeed, studies on the distribution of neutralizing antibodies among different age groups in the USA indicate that sera from children aged 2 to 4 years neutralized about 10% of the rhinoviruses investigated (a total of 56 rhinoviruses; rhinovirus 1A-55) whereas sera from adults 30 to 40 years of age neutralized just over half of the rhinoviruses investigated [48,86]. It is likely that measures of antibody prevalence by more sensitive assays such as ELISA will reveal a much higher proportion of individuals with antibody to rhinoviruses [7].

Rhinovirus infection in families

The family unit is a major site for the spread of rhinoviruses. Young children and primarily those less than 2 years of age were identified as the major introducers in Seattle [57,58]. In Virginia, the school-aged child was a more common introducer and adults were also prominent. Family size is directly related to number of family episodes of rhinovirus infections [57]. Secondary infections appearing at 2 to 5-day intervals, are most common among young children and mothers but occur in all members of the household [57,58]. Secondary attack rates in the family have varied between 30% and 70%, but most studies have revealed rates of about 50%. When antibody-free (susceptible) members only are considered, secondary rates of 70% are common. Secondary infection rates in families fall with increasing age and are higher among mothers than fathers, presumably because of their close exposure to children in the family.

Characteristics of the introducer influence the secondary attack rates in families. Among Seattle families, the secondary attack rate was 71% if the introducer was ill but only 27% if he/she was well, a finding similar to that noted for spread between spouses [57].

Rhinovirus infection in schools

Efficient spread of rhinoviruses among children in a nursery school was demonstrated by Beem [13]. Rhinoviruses clearly spread among university students, medical students, boarding school populations, and probably other groups in dormitory-type situations where there is little or no contact with children [88,103,104,164]. The finding that illness peaks were simultaneous amongst adults with and without children, has been interpreted as suggesting that something other than the opening of school in September accounted for the early winter peak in prevalence [108]. However, attempts to identify a correlative meteorological event were not successful. It is in fact quite possible that the primary determinant of a community's prevalence is indeed the attendance of children at school, and that the peaks observed in adults without children are a result of secondary spread through the community as a whole, while the peaks observed in adults with children are more likely to be a result of direct spread from children within the family.

A recent study has identified very strong correlations between rhinoviral infections and school attendance, and suggests that although other factors may play a part, attendance of children at school is a very powerful determinant of the community load of infection in both adults and children [125] (Figures 1.1 and 1.2). The prime influence presumably is infection in children crowded together at schools, with secondary attacks within and then without family units accounting for the passage of infections throughout the community.

Geographical distribution and seasonal pattern of rhinovirus infection

Rhinovirus infection is distributed worldwide [20], having been detected in the USA in many

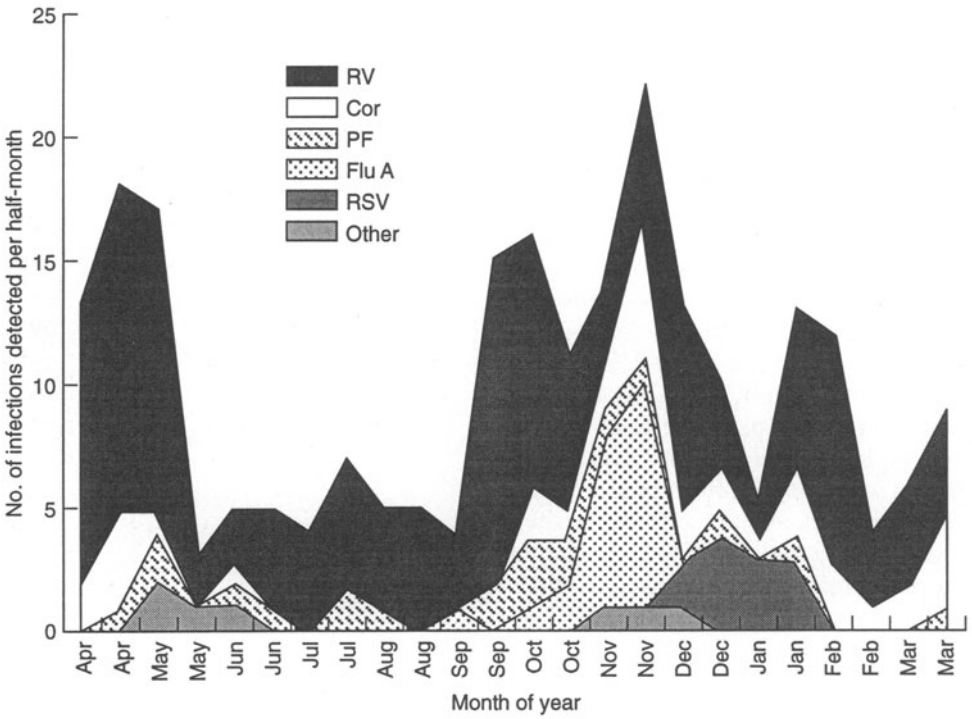

Figure 1.2 The seasonal variation in numbers of upper respiratory tract infections for individual organisms per half-month detected in the Southampton cohort of children in the year April 1989 to March 1990. Individual organisms are identified as shown: RV, rhinovirus; Cor, coronaviruses 229E and OC43; PF, parainfluenza viruses types 1–3; Flu A, influenza type A; RSV, respiratory syncytial virus. (Adapted from [125].)

studies [57,137]; in the UK [126]; in eastern Europe [101]; in a variety of South and Central American countries, including Panama with an epidemiological pattern similar to that reported for the USA [166,169], Amazon Indians [216] and Brazil [178], where almost continuous infection has been documented in very crowded impoverished urban surroundings [6]; and in isolated communities such as Antarctica [227], the bushmen of the Kalahari, Canadian Eskimos and natives of Tristan da Cunha [20,218].

Data from the USA suggest two main seasonal peaks – one major peak during late summer and early autumn (August to October), and a second peak in spring (April or May) [104,133]. In the tropics, the peak of rhinovirus infection occurs during the rainy season [169]. Schools, nurseries and family groups are the major sites for the spread of rhinoviruses in any community, and in a recent study in the UK, school attendance appeared to be the main determinant of seasonal distribution [126].

Epidemiology of serotypes

Initial observations on serotyping of rhinovirus isolates indicated that a multiplicity of serotypes existed and that surveillance of a population for at least several months would result in isolation of several distinct types. Officially numbered serotypes now extend to 100 [100], while over 115 serologically distinct

serotypes have been described. Prevalent serotypes vary from year to year, but at any one time, a small number of the serotypes tend to cause most of the illnesses [55,58,165,168]. The distribution of serotypes recovered over 7 years in Virginia changed, with an increase in higher-numbered serotypes and in non-typeable strains with time [22]. Analysis of serotypes occurring at New York, Seattle, and Michigan during this same period produced similar findings [58]. These findings suggested an emergence of new strains with time, probably as a result of selection pressure from immune surveillance.

Antigenic characterization of recent isolates, however, indicates that rhinoviruses identified earlier are still prevalent. Antisera to types 1–89 identified 15 of 16 (94%) isolates in Boston in 1982–1983, 752 of 790 (95%) isolates in Seattle in 1975–1979, and 194 of 209 (93%) isolates in Tecumseh, Michigan in 1976–1981 [100].

1.4.2 CORONAVIRUSES

Virus types

There are two major serotypes of human coronaviruses, designated 229E and OC43. Epidemiological studies of these viruses have been limited as they are very difficult to identify. Antigen for enzyme immunoassays has very limited availability, and virus culture is also difficult, as 229E will only grow in a specially adapted cell line called Clone 16, while OC43 can only be propagated reliably in suckling mouse brain.

It is probable that there are other similar but distinct coronaviruses, as the limited epidemiological studies that have been carried out have detected some viruses with atypical features; for example OC43-like viruses that have grown in cell culture, or antibodies to coronaviruses that react to both serotypes in enzyme immunoassays.

The recent development of PCR assays for the detection of both virus types should improve detection rates in future epidemiological studies [176].

Mode of transmission

There are few data available specifically on the transmission of coronaviruses, but such epidemiological data as do exist suggest that they follow the same general pattern as other respiratory viruses, in that droplet spread is the major route of transmission, and crowding of pre-school and school children the most important influence on the incidence of infection.

All the well-characterized human coronaviruses have been transmitted in human volunteers by nasal inoculation [15,16,197]. All have caused clinical illness manifest chiefly by nasal symptoms. The incubation period is around 3 days, and the duration of illness about 7 days.

Clinical features

Human coronaviruses are thought to cause around 15% of acute upper respiratory tract infections, and are also implicated in lower respiratory disease such as exacerbations of asthma [21,85,125,126,207]. However, a recent Southampton, UK survey found that of upper respiratory viral infections, those with coronaviruses were less likely to result in lower airway symptoms than upper respiratory infections with the other respiratory viruses as a group [126].

The main symptoms are rhinorrhoea, sore throat, cough, malaise and fever. Pneumonia and a pleural reaction were observed in 33% of military recruits with coronavirus infection [230].

Minor illness appears to occur throughout life, and mortality at any age is exceedingly rare.

Prevalence, age and sex distributions

Antibody to both the OC43 and 229E viruses appears in early childhood and increases in

prevalence rapidly with age [154]. The incidence of coronavirus infection among subjects with upper respiratory illness varies markedly from one season to another. In a 6-year study of 229E infections among medical students, Hamre and Beem [102] found a low incidence of 1% in 1964–1965 and a high of 35% in 1966–1967. An infection rate of 34% was found in Tecumseh, Michigan during the same peak year [170], and a rate of 24% in Bethesda, Maryland [134].

Serosurveys of OC43 infection have shown quite similar incidence rates. During peak seasons, 25% [170] to 29% [154] of adult colds could be associated with OC43 infection; overall, 17% of individuals develop antibody rises each year. In a single study in children, rates were lower, with a peak infection rate of 19% in one winter outbreak of respiratory disease but an overall winter rate of only 5% [135].

Infections seem to occur throughout life with no particular predilection for either the young or the old. No significant difference in the incidence of infection between the sexes has been demonstrated.

A recent study has found that the use of PCR increased the detection rate for both serotypes by about two-fold over cell culture and ELISA combined. This study found coronaviruses accounted for 13% of proven viral respiratory infections in a year-long survey of 9 to 11-year-old children in Southampton, UK [126].

Seasonal distribution

Both OC43-like and 229E-like viruses are epidemic, with peak incidence in the winter or spring and well-defined outbreaks. Monto has noted that 229E-like strains have appeared to cause nationwide outbreaks in the USA at roughly 2-year intervals whereas OC43-like outbreaks have been more localized [167]. Serosurveys of 229E and OC43 infections correlated with respiratory disease have not been performed since the early 1970s, so recent information on this point is lacking.

The recent study in Southampton employing cell culture, ELISA and PCR to detect both serotypes revealed mini-peaks of infection in November and April, but there was a background of endemic infection throughout the winter months [125,126] (Figure 1.2).

Geographical distribution

Antibodies to human respiratory coronaviruses have been found in all areas of the world where they have been sought, including North and South America, and Europe. Of normal healthy adults in England in 1976, 100% and 94% had antibody by ELISA to the OC43 and 229E virus types, respectively [105]. This was similar to the prevalences of 87% and 86%, respectively, that were found in adult sera collected in the winter of 1981 in southern Iraq and tested at the same time by the same method [105].

1.4.3 RESPIRATORY SYNCYTIAL VIRUS

Types of virus

Analysis with monoclonal antibodies has allowed the separation of RS viruses into two subgroups, designated A and B. This antigenic polymorphism has been shown to be largely due to variability in the G surface glycoprotein, and has remained relatively stable throughout the last 30 or more years.

Clinical illness

Most individuals infected with RS virus have upper respiratory illness. Around 25–40% of infants between 6 weeks and 6 months of age infected with RS virus for the first time, will develop bronchiolitis or pneumonia, with the peak incidence at 2 months of age [70,185]. RS virus is the major cause of bronchiolitis during early infancy and one of the major causes of pneumonia during the first few years of life. Mortality is low in modern westernized society, occurring in less than 2% of hospitalized patients. Mortality rises in the presence of

other cardiovascular or respiratory disease, and is higher in the less developed world. Reinfection is common, may occur at all ages, and is usually symptomatic. In adults RS virus normally causes simple upper respiratory infection, but cases of severe pneumonia (especially in the elderly) have been documented.

Mode of transmission and nosocomial infection

As with other respiratory viruses, RS virus is spread by infected respiratory secretions. However, the major mode of spread appears to be by large droplets or through fomite contamination rather than through droplet nuclei or small-particle aerosols [92,95]. Spread requires either close contact with infected individuals or contamination of the hands and subsequent contact between fingers and nasal or conjunctival mucosa ('self-inoculation').

RS virus is a major cause of nosocomial infection [71,94], and is a particular hazard for premature infants, infants with congenital heart disease or bronchopulmonary dysplasia, and infants and children who are immunodeficient. The rate of hospital-acquired infection for infants and children during an RS virus season ranges from 20% to 40%, and is associated with appreciable mortality in those with concurrent disease [96,152]. Hospital staff appear to play a major role in nosocomial spread through exposure to infected caregivers and transmission of fomites on hands. The likelihood of nosocomial RS virus infection increases with the duration of stay and the number of individuals housed in a patient's room [209].

Nosocomial spread in nurseries and paediatric wards can be reduced by a combination of measures that include (a) limitation of visitors, (b) active surveillance for RS virus infection among patients and new admissions, (c) cohorting of RS virus infected patients, and (d) institution of various barriers to virus spread [225], such as strict hand washing and the use of gloves and gowns [149,156].

Seasonality and geographical distribution

RS virus has a clear seasonality in temperate zones of the world. In urban centres, epidemics occur yearly in the winter months but not during the summer [139,174,231]. In the northern hemisphere, the virus is rarely isolated during August or September. Each RS virus epidemic lasts approximately 5 months, with 40% of infections occurring during the peak month in the temporal centre of the outbreak [17]. The peak may occur as early as December or as late as June, but most outbreaks peak in February or March.

The behaviour of RS virus in tropical or semi-tropical areas is somewhat different, with epidemics occurring during rainy seasons [210,215]. In Calcutta, epidemics have been found to occur during religious festivals when large groups of people gather together and overcrowding is common [113].

RS virus has a worldwide distribution and, throughout the world, is one of the most if not the most important paediatric viral respiratory tract pathogen.

Socioeconomic status, age and gender

Hospitalization for RS viral disease is more frequent in children from families with lower socioeconomic status and in heavily industrialized areas [76,205]. Hospitalization rates in lower socioeconomic groups are around 1 in 70 infections [79,80], while in middle- and high-income families, the rate is only one in 1000 infections [74].

Serological surveys indicate that infection with RS virus is almost universal during the first few years of life. Attack rates of around 50% have been noted in most epidemics [80,185,205], though in day-care centres the attack rate approaches 100% during epidemics [106]. The infection rate for RS virus is 69% during the first year of life and 83% during the

second year; virtually all children are infected by 24 months of age and 50% are re-infected at least once, the risk of re-infection decreasing to 33% by 48 months of age [80]. In seronegative infants and young children, 98% were infected with RS virus when exposed during an outbreak, and the re-infection rates were 74% and 65% during two subsequent epidemics [106]. Re-infection of adults is also common, particularly when exposure to virus is heavy. In hospital staff on paediatric wards, infection rates of 25–50% have been observed during epidemics [93,94].

Serious lower respiratory tract disease occurs 30% more commonly in infant males than in females [185], perhaps as a consequence of males having smaller airways [29].

Epidemiology of serotypes

The two subgroups of RS viruses have remained stable, and have co-circulated during most yearly epidemics that have been studied, with subgroup A normally predominating [1,110]. The impact of subgroup antigenic variation on the epidemiology of RS virus appears to be limited. Infants undergoing primary RS virus infection develop a broad serum neutralizing antibody response. Thus, although antigenic dimorphism probably contributes to the high incidence of initial re-infection during early childhood, its effect appears to be modest, especially as many re-infections involve viruses of the same subgroup [173].

Lower respiratory tract disease and mortality

Lower respiratory tract disease, principally bronchiolitis and pneumonia, are common during RS virus infections, particularly in infants. Some 40% of institutionalized infants developed pneumonia during an outbreak of RS virus infection [130]. In family studies, 25% of infants undergoing their first infection with RS virus were considered to have lower respiratory tract disease [80].

Rates of hospitalization of infants for RS virus infections vary with the setting. In Washington, DC, one in 200 infants required hospital care for RS virus pneumonia or bronchiolitis during the first year of life [139]; in Norway, hospitalization was required for one of 100 infants under 1 year of age [182], and in Great Britain, one in 120 [28]. RS virus is detected in around 50% of hospital admissions with a diagnosis of bronchiolitis, in 25% of those with pneumonia, in 11% of those with bronchitis, in 10% of those with croup and in 5.4% of infants and children seen in the clinic or admitted to hospital for non-respiratory illness [139].

Death due to RS virus infection is not common in developed countries. There are no accurate estimations of the overall death rate, but several surveys of hospitalized children with RS virus infection report a fatality rate of 0.5–2.5% [28]. Death occurs most often in infants with underlying illnesses, with a mortality rate of 37% in children with congenital heart disease, the highest mortality being in those with pulmonary hypertension [152]. High mortality has also been observed in infants and children who are immunosuppressed for treatment of other diseases such as cancer [97]. Several cases of severe or fatal disease have been described in children with disorders of cell-mediated immunity [160]. Infants with bronchopulmonary dysplasia are also at high risk for severe or fatal illness [83].

1.4.4 INFLUENZA VIRUSES

Virus types

There are three serotypes of influenza viruses that cause disease in humans: types A, B and C. Type A is by far the most common, and causes the vast majority of clinical illness related to influenza viruses.

Influenza virus type A is further divided into subtypes depending on the antigenic composition of the major surface antigens, haemagglutinin and neuraminidase (see

Chapter 13). The subtypes are designated H and N numbers to classify them. For example the first human influenza virus isolated in 1933, and that which caused the devastating 1918 pandemic, was designated H1N1, while the most recent epidemic in 1989–1990 was related to type H3N2 [26].

The influenza viruses are unique among the respiratory tract viruses in that they undergo significant antigenic variation. Both of the surface antigens of the influenza A viruses undergo two types of antigenic variation: antigenic drift and antigenic shift. Antigenic drift involves minor antigenic changes in the haemagglutinin (H) and neuraminidase (N), while shift involves major antigenic changes in these molecules.

Clinical illness

Influenza infections can cause a broad spectrum of clinical illness, varying from mild upper respiratory tract disease to life-threatening pneumonia (see Chapter 14). The severity of illness will be determined by a variety of host, viral and environmental factors, but a powerful influence is the degree of immunity. This will be determined to a large extent by factors such as previous exposure and the extent of antigenic variation from previously experienced viruses.

Antigenic drift in the haemagglutinin and neuraminidase molecules

The haemagglutinin is the major surface antigen of influenza virus and is type specific. Antigenic drift occurs in types A, B and C virus haemagglutinins but is most pronounced in human influenza A strains. After the appearance of a new subtype, antigenic differences between isolates can be detected within a few years using antisera, and studies with monoclonal antibodies indicate that minor antigenic heterogeneity is detectable among different influenza virus isolates at any time [183,206,213]. Antigenic differences have been detected among the haemagglutinins of H2N2 viruses isolated in 1957 and among the H3N2 viruses isolated in 1968. Analysis of influenza B viruses indicates that antigenically distinguishable viruses co-circulate during an epidemic [228]. Studies during an outbreak of influenza B in a boarding school in England revealed marked antigenic microheterogeneity, suggesting that antigenic drift occurs during an outbreak [183].

Antigenic drift also occurs in the neuraminidase of influenza viruses [40,184], and has been correlated with differences in amino acid sequences [30]. The frequency of isolation of N variants in *in vitro* systems is similar to that for the H, and sequence analysis shows single amino acid substitutions in the molecule at similar sites to those occurring in antigenic variants of the N that appear in the new epidemic strains arising in the human population [148].

Mechanism of antigenic drift

Antigenic drift clearly occurs by accumulation of a series of point mutations. However, single amino acid changes frequently have little effect on the antigenic properties of the surface protein, raising the question of how variants with epidemic potential arise in nature. Sequence analysis of naturally occurring drift strains reveals point mutations in two or more of the epitopes of the haemagglutinin, suggesting that in nature two or more mutations must be acquired before a new strain emerges that is able to escape neutralization by the antibody induced by preceding viruses. Since it is unlikely that two mutations simultaneously occur in one virus, it is likely that the mutations occur sequentially [226].

Nature of antigenic shift

Antigenic shifts in type A influenza viruses have occurred in 1957 when the H2N2 subtype (Asian influenza) replaced the H1N1 subtype, in 1968 when the Hong Kong (H3N2) virus appeared, and in 1977 when the H1N1

virus reappeared. All these major antigenic shifts in the virus occurred in China and anecdotal records suggest that previous epidemics also had their origin in China. Serological and virological evidence suggests that since 1890 there have been six instances of the introduction of a virus bearing a haemagglutinin subtype that had been absent from the human population for some time. For the haemagglutinin, there has been a cyclical appearance of the three human subtypes with the sequential emergence of H2 viruses in 1890, H3 in 1900, H1 in 1918, H2 again in 1957, H3 in 1968, H1 in 1977 and most recently H3 again in 1990.

The mechanisms through which these new human viruses emerge are uncertain, but there is evidence that the changes may be derived from genetic re-assortment in humans, or from genetically re-assorted animal or avian viruses [38,229]. Genetic and biochemical studies suggest that the 1957 and 1968 strains arose by a process of genetic re-assortment [72], with the new H3 in 1968 probably being donated by an avian influenza A virus [138]. However, the donating virus could have been one that contained H3 that had persisted unchanged since the 1900 epidemic. The lack of antigenic shift in influenza B and C viruses may be due to the absence of a significant gene pool in mammals or birds.

A second explanation for the origin of pandemic viruses is that the 'new' virus which may have caused an epidemic many years previously remains hidden and unchanged in some unknown place since that time. The strain of H1N1 which appeared in Anshan in northern China in May of 1977 and spread to the rest of the world, appears to be identical in all genes to the virus that caused an influenza epidemic in 1950 [177]. The most likely explanations for the prolonged absence of this virus include preservation in a frozen state or preservation in an animal reservoir. The animal reservoir option is a possibility for H3N2 viruses, since they have been found in pigs many years after they disappeared from humans [203]. However, the RNAs of animal influenza viruses are very variable [114], making it unlikely that a human strain would be conserved in all genes for many years. The absence of antigenic drift over a number of years is also difficult to explain. The third way in which new viruses could appear in the human population would be if an animal or bird virus became infectious for humans: the transmission of swine influenza viruses to humans has been documented [114].

Transmission of influenza A virus in humans

The virus is maintained in humans by direct person-to-person spread during acute infection. Isolated communities become infected by the introduction of virus by an infected individual often resulting in an explosive, but discrete, epidemic. Subsequent epidemics are initiated by re-introduction of virus [19]. Influenza virus activity can be detected in a large population centre during each month of the year [62,140]. On a global scale, influenza viruses are isolated from humans during most months of the year somewhere in the world. Influenza virus epidemics tend to occur over the winter months, as with other respiratory viruses.

Pre-school and school-age children are major vectors in the transmission of influenza A viruses in the community [63,91]. After the introduction of the Asian influenza A virus (H2N2) in 1957 and the Hong Kong virus (H3N2) in 1968, epidemics occurred soon after schools opened in September and October [128]. As with other respiratory viruses, the crowding that occurs in schools favours the rapid spread of virus by aerosol transmission [27]. The incubation period for influenza viruses is about 3 days for influenza A virus and 4 days for influenza B viruses [64].

Factors influencing the size of an epidemic

Immunological factors clearly influence the size of an epidemic: in 1957 a pandemic was

caused by an H2N2 virus to which the vast majority of the population was fully susceptible, as they had not had previous experience with influenza A viruses bearing a related H or N antigen. During 1968, the first year of spread of the Hong Kong (H3N2) virus, mortality was about one-half that caused by the H2N2 virus during its first year of prevalence. This dampening of the first H3N2 epidemic was a consequence of partial immunity induced by prior infection with H2N2 viruses that possessed a related N antigen. The size of epidemics occurring during interpandemic periods is always smaller than that of those occurring during the introduction of a new virus subtype. There is no regular periodicity in the occurrence of epidemics, indicating that the appearance of an epidemic in any given year represents a subtle interplay between the extent of antigenic drift of the virus and the waning immunity in the population. The time course of an epidemic is usually a sharp, single peak of virus activity, which then wanes as virus ultimately disappears from the population.

Morbidity

Influenza viruses have a major impact on morbidity leading to increases in hospitalization and medical consultations. High rates of hospitalization occur in the elderly, and in children less than 5 years of age with the highest rates in children less than 1 year of age [193]. Morbidity is also highest where immunity is lowest, such as in infants and young children undergoing first infection [193], and in all age groups following the introduction of new pandemic viruses. Age at the time of infection appears to be another factor that contributes independently to morbidity, as at each level of antibody to the H, infection in adults is more often asymptomatic than in children [63,75]. Infection rates and attack rates reflect a delicate interplay between the immunity in the population and the relative virulence of the virus.

Immunity to influenza viruses is incomplete, and morbidity following re-infection with the same strain or with a drift strain does occur, although it is usually less severe [44,45,62,65,66]. Significant morbidity and mortality with influenza A viruses can occur during nosocomial spread of infection [71,90].

Mortality

Appreciable mortality associated with influenza B virus has been seen in only two influenza seasons, 1961–1962 and 1979–1980, whereas influenza A has caused appreciable mortality in well over 20 epidemics since 1934 [180,181]. The highest mortality rates occur in people over 65 years of age [4,11,151]. However, mortality is seen at all ages; during the 1918 epidemic, the mortality rate was extremely high in young adults as well as in those over 65 years of age. The coexistence of cardiovascular, pulmonary, metabolic or neoplastic disease and pregnancy all increase the risk of mortality [9,10,82]. In epidemic years such as 1957–1958, the mortality totalled 70 000, and 1 in 300 of adults over 65 years of age died from influenza infection. Although excess mortality is highest during the first year of circulation of a new subtype, the cumulative interpandemic mortality significantly exceeds that caused during the first year [180].

Strain differences

The clinical illnesses caused by influenza A viruses belonging to different subtypes are remarkably similar. There are, however, some differences between virus strains in both the illnesses they cause, and in their epidemic behaviour [140,225,232]. H3N2 viruses are more frequently associated with croup, and the H1N1 virus that caused the 1918 pandemic was associated with very high mortality in young adults, an age group that was not at high risk for mortality in subsequent H1N1, H2N2, or H3N2 epidemics [225]. It is also likely that a variety of host, viral and environmental factors

play a role in this variability. Encephalitis lethargica has been associated with the early H1N1 virus, but not with any others [196], while the H1N1 virus introduced in 1977 caused a relatively mild illness in susceptible children and young adults who would not have encountered it previously [69,180,232].

Immunity

Immunity to influenza viruses is primarily mediated by responses to the surface glycoproteins, is long-lived and is subtype-specific [63,68,73,180,194]. People infected previously with H1N1 viruses which circulated between 1918 and 1957 were resistant to infection or disease in 1977. The duration of immunity in the presence of antigenic drift has been estimated to be between 1 and 5 years depending on the extent of the drift [65,66].

Although immunity can be long-lived, reinfection with homologous influenza A viruses occurs, indicating that immunity induced by a single infection can be incomplete [65,66]. This incomplete immunity is likely to be a result of a gradual diminution in local and serum antibodies within the first year following first infection [123]. In addition, infection with a new influenza virus subtype leads to generation of antibodies that react with only a limited number of antigenic sites, whereas after second or subsequent infections, antibodies with a broad range of specificities are produced.

1.4.5 PARAINFLUENZA VIRUSES

Virus types

There are four serotypes of parainfluenza viruses causing disease in humans, designated types 1–4, with type 4 being subdivided into subtypes 4A and 4B. Each of the four parainfluenza virus types can cause acute respiratory tract disease in humans.

Mode of transmission

Transmission of parainfluenza viruses is by direct person-to-person contact or by droplet spread, but the viruses do not persist in the environment. All four serotypes have been transmitted in experimental infection studies, and cause upper respiratory tract symptoms [132,219,220]. The high rate of infection early in life, coupled with the frequency of re-infection, suggests that the viruses spread readily from person to person. The type 3 virus appears to be the most efficient in its ability to spread, and generally infects all susceptible individuals in an enclosed population in a relatively short time, while types 1 and 2 are less effective, infecting 40–69% of susceptible individuals [25,131]. Parainfluenza viruses are introduced into the family primarily by preschool children.

In experimental infection of adult volunteers, the interval between administration of type 1, 2 or 3 virus and onset of upper respiratory tract symptoms ranges from 3 to 6 days [132,208,219,220]. The interval between exposure to type 3 virus and subsequent viral shedding is 2–4 days [25]. Type 3 virus is shed from the oropharynx for 3–10 days (median 8) during initial infection, and for a shorter period during re-infection [25]. However, prolonged shedding in infants and young children has been observed for as long as 3–4 weeks [67]. Prolonged shedding of type 3 virus has also been observed occasionally in adults with underlying chronic lower respiratory tract disease [84].

Clinical course of parainfluenza virus infections

All four serotypes of parainfluenza viruses cause acute respiratory illness in humans, with the most common symptoms being rhinitis, pharyngitis, bronchitis and fever [186,187].

Parainfluenza virus type 1 is the principal cause of croup (laryngotracheobronchitis) in children, and parainfluenza virus type 3 is second only to RS virus as a cause of pneumonia and bronchiolitis in infants less than 6 months of age [24,25,46,186]. Parainfluenza

virus type 2 resembles type 1 virus in the clinical manifestations it causes, but serious illness occurs less frequently; infections with type 4 parainfluenza virus are detected infrequently, and associated illnesses are usually mild.

The parainfluenza viruses are most important as respiratory tract pathogens during infancy and childhood, when types 1, 2 and 3 viruses can cause anything from asymptomatic infection to life-threatening lower respiratory illness. In addition to croup, types 1, 2 and 3 viruses are also responsible for a smaller but appreciable proportion of other acute respiratory tract diseases of infancy and early childhood.

Geographical distribution

Parainfluenza viruses have a wide geographical distribution. The first three types have been identified in most areas where appropriate diagnostic techniques have been used to investigate childhood respiratory tract disease. Type 4 viruses, which are more difficult to culture, have been isolated in fewer areas, but serological studies suggest that they are as prevalent as the other types.

Prevalence of parainfluenza virus infections

The parainfluenza viruses are exceeded only by RS virus as an important cause of lower respiratory tract disease in young children, and they commonly re-infect older children and adults to produce upper respiratory tract disease. Re-infection of adults as well as children has been recognized on a number of occasions, particularly with type 3 virus. Although the frequency of re-infection is not known, it is probable that most individuals have repeated infections with types 1, 2 and 3 viruses. In a study of three outbreaks of type 3 virus infection in a nursery population, 17% of the children infected during one outbreak were re-infected during a subsequent outbreak, although the interval between the first and last outbreaks was only 9 months [25]. Re-infection of pre-school children living at home also occurs with high frequency [77]. Illness usually occurs less often (and is less severe) during re-infection than during primary infection.

Type-specific infection rates are difficult to estimate because there is considerable cross-reactivity between the serotypes. Family studies reveal rapid spread of virus within infected families, with 64% of family members developing a serum antibody response.

Age distribution

Parainfluenza infection generally occurs very early in life. Serological surveys indicate that at least 60% of children are infected with parainfluenza type 3 virus by 2 years of age and that over 80% are infected by 4 years of age [178,179]. Longitudinal studies suggest that serological surveys may underestimate the prevalence [77]. Infection with type 1 or type 2 virus generally occurs somewhat later in childhood, but by 5 years of age a majority of children have been infected with type 2 virus, and over 75% have been infected with type 1 virus [186,187].

Type 3 virus often causes illness during the first months of life at a time when infants still possess circulating neutralizing antibodies from their mothers. In contrast, in young infants, maternally derived antibodies appear to prevent both infection and severe disease with either type 1 or type 2 virus [24,25,76]. After 4 months of age, there is a rise in the number of cases of croup and other lower respiratory tract diseases caused by type 1 and type 2 viruses. This high incidence continues until approximately 6 years of age.

After school-age, there is a much lower incidence of severe lower respiratory tract disease caused by type 1 and type 2 parainfluenza viruses, and the occurrence of lower respiratory tract illness in individuals infected with either virus during adolescence or adult life is unusual, although this does occur on occasion. Parainfluenza viruses have been associated

with exacerbations of asthma in several studies [126,191].

Seasonal pattern

Some investigators have shown parainfluenza virus infections to follow an endemic pattern, with infection occurring sporadically and without a definite seasonal pattern [24,25,125] (Figure 1.2), while others have shown sharp autumn outbreaks occurring in 2-yearly cycles [25,78].

1.4.6 ADENOVIRUSES

Virus types

There are over 50 serotypes of adenovirus described so far, and new strains continue to be identified, particularly in the immunocompromised patient.

Serologic surveys have shown that antibodies to types 1, 2 and 5 are most common and are present in 40% of children [18,129]. The incidence of antibodies to types 3, 4 and 7 is low at the same ages. These antibody results probably explain why adults are more commonly infected with types 3, 4, and 7. During the surveillance for the Virus Watch studies it was documented that only about 75% of the adenovirus isolates were accompanied by an antibody response, as measured by complement fixation [56]. The reason for some of the serological non-responders may have been the insensitivity of the assay or the failure of some children to produce such antibodies.

Clinical illness

Adenovirus infections can cause a wide variety of clinical illnesses including asymptomatic infection, upper respiratory tract diseases, pharyngo-conjunctival fever, severe, life-threatening pneumonia, and enteric and hepatic infections.

Approximately 5% of acute respiratory disease in children under the age of 5 years is thought to be associated with adenoviral infection [18]. The most frequent symptoms are nasal congestion, coryza and cough, though exudative pharyngitis may also occur. Systemic symptoms such as fever and myalgia are common.

Adenoviruses are thought to account for about 10% of cases of childhood pneumonia. Most children will recover, but mortality has been considerable in some epidemics, and it is thought that bronchiectasis may follow severe infection early in childhood [145,204].

Geographical distribution

Adenovirus infections occur worldwide in humans. The transmission of infection and disease varies from sporadic to epidemic. The pattern often correlates very well with the viral serotype and the age of the population. Adenoviruses probably account for 3% of respiratory infections in civilian populations and approximately 7% if only febrile illnesses are considered [56]. The corresponding figures for young children are approximately 5% and 10%, respectively. However, in the same studies a large number of completely asymptomatic adenovirus infections have also been documented.

Mode of transmission

Faecal–oral transmission accounts for many infections in young children. Initial spread may occur via the respiratory route, but the prolonged carriage in the intestine makes the faeces a more common source during both the acute illness and intermittent recurrences of shedding [56]. Controlled studies of routes of infectivity for adenoviruses that caused epidemic acute respiratory disease among military recruits have demonstrated that aerosolized virus inhaled into the lungs of volunteers produced the disease, whereas application to the mouth, nasal mucosa or intestine did not [36].

Several of the adenovirus serotypes have probably also been spread as nosocomial infections [14,224].

Epidemic adenovirus infections in military recruits

Epidemics of acute respiratory disease were well known during World War II, and this awareness preceded the isolation and the characterization of the first adenovirus by approximately one decade. However, the results of later studies suggested that adenovirus type 4 or 7 infection caused most outbreaks [37,111,112]. These epidemics occurred almost exclusively in recently assembled military recruits, and were most common in winter. They did not occur in seasoned personnel in close contact with the recruits, suggesting that seasoned personnel had some form of immunity. However, the disease did not occur in similarly congregated college students, suggesting that more crowded sleeping conditions or the fatigue associated with training were contributing factors. The importance of these cofactors was supported by the observation that the adenovirus infections did not spread to civilian personnel in contact with the military. Adenovirus-induced respiratory disease often affected 80% of the recruits, with 20–40% hospitalized.

1.4.7 ENTEROVIRUSES

Virus types

The major human enteroviruses include poliovirus types 1–3, echoviruses [31 serotypes), Coxsackie viruses, of which there are two major groups, A and B, with many different serotypes within each group and at least five other known enteroviruses. Many are capable of causing acute respiratory illness, as well as gastrointestinal illness for which they are named. They share many structural and biological similarities with human rhinoviruses.

Mechanisms and routes of transmission

Humans are the only known reservoir for members of the human enterovirus group, and close human contact appears to be the primary mechanism of spread. Almost all enteroviruses can be recovered from the oropharynx and intestine of individuals with symptomatic and asymptomatic infections. As with other respiratory viruses, droplets or aerosols from coughing or sneezing are the major source of transmission when the infection is upper respiratory (faecal–oral spread is important for enteric infections). Coxsackievirus A21 has been transmitted experimentally from infected volunteers by airborne aerosols produced by natural coughing [37].

Family studies

Outbreaks of aseptic meningitis and other related illness due to echovirus 30 began to spread along the Pacific Coast of the USA in 1968, and its arrival in Washington State coincided with the initiation of the Seattle Virus Watch Program, thus permitting close observation of infection and illness among the regularly studied Virus Watch families [99]. A total of 64 families containing 291 members were studied with continuing virus isolation and serology; infection was documented in 70 (79%) of 88 members of 18 families; in the total observed Virus Watch population, the rate was 24%. The affected families tended to be of larger size, included more children 5–9 years of age, and included only three persons who had antibody before the epidemic. Mild febrile illness was reported in only 47% of those shedding virus; few of these episodes were serious enough to require medical attention, and only one subject developed aseptic meningitis. Extending these observations to the general population suggests that there must have been many thousands of echovirus 30 infections in the area, more than half of which were without symptoms, during a period when 44

virologically confirmed cases of echovirus 30 aseptic meningitis occurred in the city [217].

Geographical distribution and age

Enteroviruses are found throughout the world. In tropical regions they are present throughout the year, but in temperate climates there is a late summer, early autumn peak [172]. It is well recognized that enteroviral infections are more common among lower social classes and in the presence of poor hygiene. Most reported cases of enteroviral infection occur in children, with 56% being under 10 years of age, and 26% under 1 year [172].

Clinical course of enterovirus infection

Respiratory disease associated with enteroviruses is commonly upper respiratory symptoms indistinguishable from those caused by other respiratory viruses. They have also been implicated in episodes of pneumonia and bronchiolitis [201].

A survey of enteroviral disease in the USA from 1976 to 1979 revealed that meningitis was present in 35% of patients, respiratory disease in 21%, encephalitis in 11% and non-specific febrile illness in 6%, with a variety of other syndromes making up the remainder [172].

Several Coxsackieviruses and echoviruses have been associated with mild upper respiratory illness, and also occasionally with mild or severe lower respiratory disease [43,157].

1.4.8 CYTOMEGALOVIRUS

Virus type

Cytomegalovirus (CMV) is a herpes virus that is ubiquitous, infecting many animals as well as humans. Humans are believed to be the only reservoir for human CMV infection. It is thought that there may be genetically distinct strains of CMV that are continuously circulating throughout the world [3].

Mechanism of spread

Transmission occurs by direct or indirect person-to-person contact. Virus has been detected in almost all bodily fluids. However, because of the lability of CMV to environmental factors, close or even intimate contact is believed to be required for horizontal spread. The community prevalence is enhanced by the fact that virus excretion persists for years after congenital, perinatal and early postnatal infections [2,144], and prolonged replication can follow primary infection in older children and adults. Recurrent infections are also associated with shedding of CMV from many sites in a significant proportion of seropositive young adults [141,199].

Oral and respiratory spread appear to be the dominant routes of transmission during childhood and probably adulthood as well. CMV is also spread through venereal routes: in many populations there is a burst of infection with the advent of puberty, and infection rates are much increased in promiscuous populations. Multiple blood transfusions or transfusions of large quantities of blood are associated with an increased risk of both primary and recurrent CMV infection.

Clinical course of CMV infection

Intrauterine infection can cause abortion and choroidoretinitis. The vast majority of peri- and postnatal CMV infections are likely to be asymptomatic, or to present with mild flu-like symptoms indistinguishable from those caused by other respiratory viruses. CMV may also cause more prolonged and severe illness similar to infectious mononucleosis, and in the immunocompromised CMV frequently produces life-threatening pneumonitis and retinitis; it may also be implicated in episodes of graft rejection in transplanted subjects.

Prevalence, age and geographical distribution

CMV infection is endemic rather than epidemic and is present throughout the year

rather than being seasonal [81]. Climate does not appear to affect infection rates. Ill-defined socioeconomic factors do predispose to higher infection rates, both by vertical (intrauterine) and horizontal (extrauterine) transmission [81,212]. Poor hygiene alone cannot explain the higher infection rates: once again, the closeness of contacts within population groups is important. Very high rates of infection among children have been recorded in isolated locations, as well as crowded areas of Africa, the Orient and the Middle East, irrespective of hygienic practices [81]. Transmission among toddlers is exceptionally high in day care centres and boarding schools [120,188]. After infancy in most developed countries, infection rates increase slowly until the age of entry into school, at which time they rise more rapidly; 40–80% of children are infected before puberty [81]. In other areas of the world, 90–100% of the population may be infected during childhood, even as early as 6 years of age [81]. Young infants and children with subclinical infection appear to be the major source for primary infection in pregnant women [190]. Day care centres and similar settings where pregnant women are in daily contact with children, especially toddlers, pose a high-risk setting for primary infection with its increased risk for intrauterine transmission [120,189].

Recurrent and chronic infections with CMV

Recurrent infection is defined as intermittent excretion of virus from single or multiple sites for a number of years and is to be distinguished from 'chronic' or 'prolonged excretion' of virus, which characterizes certain forms of CMV infection, particularly in the immunocompromised. Recurrent infection can result from one or more of three mechanisms. First, after primary infection, a low-grade chronic infection may be established in which virus excretion reaches detectable levels only periodically. Second, re-infection may occur in immune people because of antigenic and genetic disparity among CMV strains. Third, CMV may become latent in various organs during the primary infection, as with herpes simplex virus, and be repeatedly reactivated in later life in response to different stimuli.

1.5 THE COMMON COLD

1.5.1 HISTORY

The term 'common cold' describes the universally recognized short mild illness in which the main symptoms involve the upper respiratory tract and in which nasal symptoms usually predominate. The symptoms usually comprise some or all of the following: nasal stuffiness, sneezing, coryza, pharyngitis, throat irritation, and mild fever. The name 'common cold' probably arose from a combination of the fact that the onset of symptoms included the feeling of chilliness on exposure to cold, and the increased prevalence during the winter months, giving the impression that there may be a cause-and-effect relationship. This is still a commonly (and erroneously) held view, even though as long as 200 years ago Benjamin Franklin pointed out that colds were caught from other people rather than by exposure to cold. In 1914 the infectious nature of colds in humans was demonstrated by the instillation of filtered, cell-free nasal washings from ill people with colds into the nares of subjects who subsequently developed colds. These findings were confirmed in 1930 by Dochez *et al.*, who provided the first evidence that colds were caused by a virus and not bacteria by inoculating volunteers with bacteria-free filtrates from individuals with symptoms of colds [49]. In 1933, the isolation and cultivation of an influenza A virus from a human was reported, thus opening avenues for further detailed study of common cold viruses.

Rhinoviruses were first reported in 1953 to have been cultured in explants of human embryonic lungs [5]. However, it was not until some years later that this work could be repeated when the virus was shown to pro-

duce a cytopathic effect in human embryo kidney cells [221,222]. In the meantime, two groups in the USA reported the isolation in monkey kidney cell cultures of a virus involved in upper respiratory illness, and this was subsequently designated rhinovirus 1A [192,195]. Since then, other rhinovirus-sensitive cells have been described, and over 115 different serotypes of rhinoviruses have been isolated, all of which have been found to cause common colds.

A major contribution to our present understanding of the common cold has been the use of human volunteers under carefully controlled conditions. The Common Cold Research Unit at Salisbury, England, was established in 1946, and only closed recently on the retirement of its director, David Tyrrell in June 1990. Volunteer studies here as well as those performed in the USA during the last 40 years are to a large degree responsible for our present understanding of colds in adults. Although studies of respiratory illness in children have also been extensive (in particular those concerned with the spectrum of clinical manifestations by age group, and seasonal prevalence rates of the different respiratory viruses), controlled volunteer trials have for ethical as well as practical reasons, not been performed.

1.5.2 AETIOLOGICAL AGENTS

It has been known for some time that rhinoviruses are responsible for the majority of common colds, and that coronaviruses, RS viruses, influenza viruses, parainfluenza viruses, adenoviruses, enteroviruses, and a variety of less common viruses and atypical bacteria may also cause common colds and contribute to the total disease load (see Table 1.1). These agents are all covered in detail in other chapters; in this chapter an overview of the epidemiology of each virus type has been presented.

As a group, rhinoviruses are the most common cause of colds in children as well as adults. Also of major importance in the aetiology of colds are re-infections with parainfluenza viruses and RS virus. Although relatively little data exist on the contribution of coronaviruses to colds, the data that have been collected in those studies in which they have been looked for suggest that they contribute around 15–20% of common colds [125,153,154,159,230].

1.5.3 EPIDEMIOLOGY

The common cold is a frequent illness of childhood, with prevalence decreasing with age as a result of increasing immunity and reduced opportunity for spread, itself a result of changes in behavioural patterns. Despite the fact that over 200 serologically different viral types are likely to cause this illness, there is a general predictability of incidence and seasonal occurrence.

Prevalence rates vary considerably depending on age, the time of year, year-to-year variation, and on the population being studied. An example of the seasonal pattern for individual viruses, in 9 to 11-year-old children in the UK for the year 1989–1990 is given in Figure 1.2. From this it can be seen that as a general principle, rhinoviral infections occur all-year round, but peak in early and late winter, coronaviral and parainfluenza viral infections occur all-year round, though again with peaks in winter, and that influenza and RS virus have discrete winter peaks of infection.

Although numerous epidemiological studies have been conducted on the occurrence of respiratory illnesses, it is difficult to calculate a precise incidence of the common cold because criteria of disease classification have been different in different studies, incidence varies greatly with population characteristics and age, and because viral detection methods have been only partially successful in detecting the causative agents. It has been estimated that common colds occur at rates of two to five per person per year [35,59,60], though recent epidemiological data have shown that in school-

children, the figure is more likely to be in the region of 7–10 colds per year [126], with younger children being likely to have more, and adults less, than that figure (many previous studies have suggested that adults have about half the number of colds as do children). Common colds are estimated to cost billions of dollars each year in terms of lost working days, cold remedies and analgesics [35].

The conventional spread of colds has its initial focus in the school. School-age children become infected and introduce secondary infections in the home. In these homes the secondary attack rate is highest in other school-age children and pre-school-age children, with the secondary attack rate in adult family members being half that for the children. The introduction of infection in the family by adults is unusual. Modern-day trends toward day-care centres and pre-schooling are likely to have increased primary infections in these younger children and will have made them the source from which secondary family infections frequently occur. Among children, boys tend to have more colds than girls. On the other hand, in the conventional family setting mothers tend to have at least one more cold per year than their spouses.

Colds occur throughout the world [6,20,166, 169,178,216,218,227]. In non-isolated populations, colds are more frequent during the winter months than during the summer time. In the tropics, colds are more prevalent during the rainy season. In isolated populations in which the number of people is fixed (such as members of Antarctic exploration teams or isolated island communities), colds do not occur unless introduced by a visiting person [227].

Although colds can be produced regularly in volunteers, the method(s) of transmission of viruses, which result in colds under natural circumstances, are still debated. The greatest concentration of common cold virus is in the nasal secretions. Children tend to have greater concentrations of virus than adults and they tend to shed virus for a longer period. Sneezing, nose blowing, and the general contamination of external surfaces (including the sufferer's hands) with nasal secretions are the main sources for viral transmission. The route of acquisition of virus is by the nose and possibly the conjunctiva. Susceptible individuals become infected by inhalation of virus in small-particle aerosols generated by sneezes, by direct nasal impaction of virus-containing large droplets from a sneeze, by nose blowing, or by the inoculation of virus (usually by the fingers) from nasal secretions that have been transferred directly or indirectly from infected subjects.

There is considerable folklore related to the catching of a cold. There is no evidence to date to indicate that cold weather *per se*, chilling, wet feet, or draughts play any role in the susceptibility of people to colds. Epidemiological data suggest that school attendance and other forms of crowding populations (particularly children) are the major factors influencing common cold virus transmission rates.

1.6 RESPIRATORY VIRAL INFECTIONS AND EXACERBATIONS OF ASTHMA

Over the last 30 years there has been a large number of epidemiological studies in which the association of respiratory viral infections with asthma attacks has been investigated. Although many of these studies had shortcomings in their design, and all acknowledged the likelihood of underestimating the contribution of viral infections as a result of difficulties in viral detection, they provide considerable evidence to support a positive association [191].

The identification rate for viruses during exacerbations of asthma (10–50%) is similar to that generally found during respiratory infections, and more specifically so in studies that have investigated episodes of respiratory infection with and without asthma or wheezing [116,117]. This rate is much higher than the viral identification rate generally found during

asymptomatic periods in asthmatics and non-asthmatics, which is around 3–5% [117,119, 122,163]. A recent study in children, using much more sensitive modern detection techniques, has produced similar findings, with the detection rate in colds without asthma, and colds with asthma being 80–85%, while the detection rate in the same children when asymptomatic was 12% [126].

There is also a close temporal relationship between viral infections and asthma exacerbations, both within individuals and within populations [107,153]. The rate of virus identification decreases after the acute stage of respiratory illness [116,118], which makes chance coincidence of virus identification with wheezing episodes unlikely. In three separate studies in which monitoring was intensive and specimens were obtained on a regular basis between episodes, virus identifications in individuals clearly coincided in time with asthma exacerbations [153,161,200]. Wheezing associated with infection has a characteristic pattern: in a recent study of children aged 1–6 years, the wheezing started 2 days after the first symptoms of respiratory infection, and lasted for 4 days [159], while in a study in 9 to 11-year-old children (see below), lower respiratory symptoms followed 1 day after the onset of upper respiratory symptoms, and lasted for 7 days, and peak expiratory flow recordings took longer to recover, at least 14 days [126].

A study of paediatric hospital admission rates for asthma provided indirect evidence: there was a striking relationship to school holiday periods, which were associated with troughs in admission rates, followed by a sharp rise to a peak after the beginning of each school term [214]. This pattern is similar to that observed for respiratory virus infections, and the authors suggested that viruses were acquired from other localities during holiday travel and were then rapidly spread through a susceptible population, largely as a result of crowding of the children at school. This hypothesis is supported by a recent study in which proven viral infections and asthma admissions were shown to be closely correlated in time in both adults and children [125] (see below).

The results of numerous studies have documented an association between the viral identification rate and the severity of the wheezing illness, an association that argues for a causal relationship between the two [12,118,161, 162,200].

The individual viruses most likely to be associated with wheezing attacks vary with age, but rhinoviruses, RS virus and parainfluenza viruses are the predominant organisms, although it should be noted that in only one study were methods used specifically for coronavirus [23].

The associations listed above argue for a causal link, but are not conclusive, as the viral identification rates (typically 20–30% of exacerbations) in many of the studies were low (particularly for rhinoviruses and coronaviruses), and many studies had weaknesses in design, such as lack of objective assessment of the episodes being studied, and retrospective or cross-sectional design.

Virus detection rates have been increased remarkably by the recent development of PCR-based assays for the major respiratory viruses, most particularly for rhinoviruses [121,127] and coronaviruses [176], which are difficult to detect using standard methods.

These methods have been used recently in a detailed, intensive prospective study of asthma exacerbations in 108 school-age children in Southampton, UK. In this study, detailed diary card recordings were made of both upper and lower respiratory symptoms, and there were peak expiratory flow recordings for a 13-month period from April 1989 to 1990. Using precise objective criteria to define exacerbations, it was found that viral infections were associated with between 80% and 85% of exacerbations depending upon the method of definition of the episode [126]. An example of the asthma

exacerbations studied and the virus infections associated with these episodes is depicted in a chart drawn of the recordings made by three of these children in Figure 1.3. The close association of the viral infections with the asthma exacerbations is clearly seen.

Rhinoviruses accounted for almost two-thirds of the viruses associated with exacerbations, with coronaviruses being next most common at 13% of viruses detected [126].

The same authors also examined the association between the seasonal pattern of viral

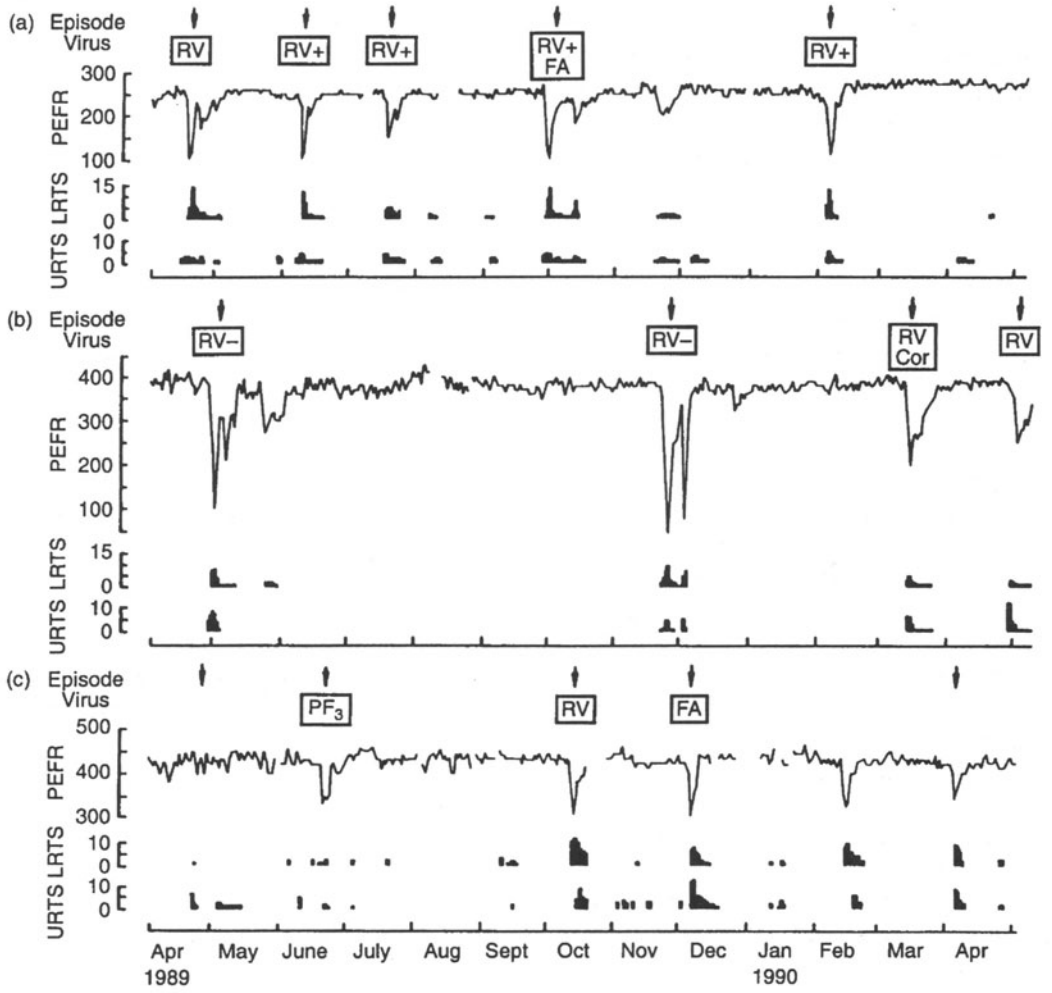

Figure 1.3 Example of charts drawn of the peak flow recordings and respiratory symptom scores for three of the children (A–C) taking part in the Southampton study of the relationship of upper respiratory viral infections to asthma exacerbations in school-age children. The horizontal axis represents the 13 months of the study. On the vertical axes, URTS and LRTS represent the upper and lower respiratory tract symptom scores respectively, PEFR represents the morning peak expiratory flow rate. Reported episodes are indicated by vertical arrows, and the viruses detected by the following symbols: RV, rhinovirus (+, major group; –, minor group); FA, influenza type A; Cor, coronavirus; PF_3, parainfluenza virus. Those reports with no such symbol had no virus detected. (Adapted from [126].)

respiratory infections in this sentinel cohort of children, and hospital admissions for asthma in both adults and children for the hospitals serving the areas from which the cohort was drawn. Strong correlations were found for both adult ($r = 0.53$; $P < 0.01$) and paediatric ($r = 0.68$; $P < 0.0001$) asthma admissions, though the relationship was clearly stronger in children [125]. Once again a profound influence of school attendance on infection rates (Figure 1.1), and therefore asthma exacerbation rates was observed, with 84% of combined adult and paediatric admissions occurring during school term times, compared with only 16% in school holiday periods [125] ($P < 0.0001$).

A similar, but less intensive study of asthma exacerbations in adults has supported these findings, with viral infections being detected in 44% of adult asthma exacerbations, a rate much higher than any previous study in adults [179].

These recent studies confirm the findings of the previous studies, and in particular confirm the importance of rhinovirus infections, which were the major virus type detected. Further studies in different age groups, geographical locations and seasons, and using equivalent methods for all the respiratory viruses are now required before a fully informed picture will be gained.

1.7 CONCLUSIONS

The main causative agents of respiratory tract infections are now known to be a large number (well in excess of 200) of respiratory viruses. Rhinoviruses are the most important virus type in school-age children and adults, while RS virus is important in pre-school-age children and infants. Influenza virus type A causes periodic severe epidemics with appreciable morbidity and mortality.

The main vectors for transmission are pre-school, and school-children, and the major factor influencing transmission rates is crowding together of children.

Detailed epidemiological studies of the Virus Watch type have provided a great deal of information with regard to the prevalence of infections, both symptomatic and asymptomatic, patterns of illness, and the influence of immunity, socioeconomic and other factors on disease prevalence. These studies were carried out with great thoroughness, but were hampered by the lack of availability of good detection methods. Many of the conclusions still hold true, particularly with regard to patterns of illness, rather than absolute numbers. The use of molecular methods of virus detection should allow a more precise picture of virus-related illness to be gained in the future.

Despite the many advances in medical science, infections caused by the majority of the agents discussed remain difficult to treat. Partial success has been achieved in the case of influenza virus type A infection, which is potentially preventable with vaccination, but there are great problems with delivery of the vaccine to those at greatest risk (the elderly, and those with chest and other serious disease), and with keeping abreast of antigenic variation.

Interest in common cold viruses has recently increased as a result of modern investigative techniques that have vastly improved our understanding of respiratory viruses, and which may in the future lead to the development of effective treatments [124].

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