

# **Viral pathogens and epidemiology, detection, therapy and resistance**

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## **Abstract**

Worldwide community-acquired pneumonia (CAP) is one of the most frequent infectious diseases and a leading cause of death. Several studies have shown that a pathogen could be identified only in 50 to 60% of all patients, although in children < 6 month infectious agents can be detected in about 90%. Viral infections are most frequent in children < 2 years (80%), whereas bacterial infections increase with age.

RSV, influenzaviruses, rhinoviruses, parainfluenzaviruses and adenoviruses are the most common viruses associated with CAP in children. Among adenoviruses a predominance of adenovirus 7 has been reported in several countries with emergence of highly pathogenic variants with significant lethality in young children. Many childhood respiratory infections are caused by more than one pathogen and up to 30% mixed viral / bacterial infections can be observed. CAP in immunocompetent adults is rare, whereas persons with underlying diseases have an increased incidence of CAP. In the elderly, RSV, influenzaviruses, parainfluenzaviruses and less frequent adenoviruses are predominant viruses causing pneumonia. Less frequently associated with CAP are the newly discovered human metapneumovirus and the coronaviruses NL63 and HKU1. Hantaviruses, involved in the hantavirus pulmonary syndrome, belong to the emerging pathogens to date in North, Middle and South America.

For optimum diagnosis the whole spectrum of potential respiratory viral agents should be included and multiple diagnostic techniques have to be used.

In view of the high relevance of influenzavirus for CAP influenza vaccination is highly advisable for prevention of CAP, especially in high-risk groups.

## **Introduction**

Viral infections are involved in 10–25% of CAP. Frequently mixed infections, viral/viral or viral/bacterial, are detected. CAP in infants and young children is most commonly due to viral infections, with the predominance of respiratory syncytial virus (RSV). In developing countries the incidence of viral pneumonia is higher and is a relevant reason for death of young children [1, 2].

## **Viral pathogens and epidemiology**

The major viral pathogens are summarized in Table 1. Usually they cause mild self-limited illness, mainly restricted to the upper respiratory tract. The leading viral pathogens for severe disease are RSV and influenzaviruses. In the past two decades some new, mostly zoonotic viral pathogens have emerged like SARS-coronavirus, avian influenzaviruses and hantaviruses associated with hantavirus pulmonary syndrome (HPS), which has crossed species barriers. Numerous other viruses occasionally can cause severe respiratory disease in the lower respiratory tract, but they are better known for other clinical manifestations (Tab. 2).

Respiratory virus infections are more common in winter and early spring, but some cause respiratory illness without a clear seasonal pattern.

The frequency of detection of respiratory viruses varies in different studies due to methodological problems. Many studies did not include all known respiratory viruses. Additionally, the prevalence of viruses may vary over time and between different geographical areas. In immunocompromised patients exogenous respiratory viruses may persist with prolonged shedding.

Individuals with a subclinical infection may be an undetectable source of transmission. Only a few studies have investigated respiratory viruses in asymptomatic humans with very variable results. An age-dependent occurrence of asymptomatic respiratory infections has been reported with the highest frequency of 68% in young children newborn to 4 years.

## **Factors influencing occurrence of community-acquired pneumonia (CAP)**

Virus-associated CAP occurs mainly in infants and young children, in elderly and frail adults, in persons with underlying diseases and in immunocompromised patients. Even “harmless” agents like rhino- or coronaviruses frequently induce acute disorders or exacerbations in people with chronic cardiopulmonary diseases or asthma. The main host and environment related risk factors have been investigated, but the influence of various other factors, which can predispose CAP is controversial.

In general, the viral-associated CAP decreases with age. In several recent studies in developed countries a pathogen was detected in 79% to 85% in immunocompetent children with CAP; 25% to 62% of the patients had evidence of a single viral infection, 8% to 11% for viral/viral infection and 23% to 43% a viral/bacterial infection. Inflammation and disease were more severe in viral/bacterial infection [3–6]. In nonimmunocompromised adults the demonstrated viral etiology is less common. Roux et al. [7] identified a pathogen only in 38% of the cases with a proportion of viral infections of approximately 20% (single viral infection 9%) with influenzavirus

Table 1. Major respiratory viruses

Virus family / Subfamily	Genus	Virus type
<i>Adenoviridae</i>	Mastadenovirus	Human adenovirus (1 – 7, 14 and 21)
<i>Coronaviridae</i>		Human coronavirus (HCoV-OC43, -229E, -NH, -NL63, -HKU1) SARS-coronavirus
<i>Orthomyxoviridae</i>		Influenzavirus A, B, C Avian influenzavirus A (e.g. H5N1)
<i>Paramyxoviridae</i>		
Paramyxovirinae	Respirovirus Rubulavirus	Parainfluenzavirus 1, 3 Parainfluenzavirus 2, 4a/b
Pneumovirinae	Pneumovirus Metapneumovirus	Respiratory syncytial virus (A, B) Human metapneumovirus (A, B)
<i>Picornaviridae</i>	Rhinovirus Enterovirus	Human rhinovirus (HRV 1-102) Human enterovirus (Cox A10, 16, 21, Cox B1-6, ECHO 4, 9, 11, 25, 32, HEV 68, 69, HRV 87)

Table 2. Other respiratory viruses

Virus family / Subfamily	Genus	Virus type
<i>Bunyaviridae</i>	Hantavirus	e.g. Sin-Nombre virus, Andes virus, Choclo virus
<i>Paramyxoviridae</i>	Morbillivirus	Measles virus
<i>Herpesviridae</i>		
Alphaherpesvirinae		Herpes simplex virus (HSV) (rare) Varicella-zoster virus (VZV)
Betaherpesvirinae		Cytomegalovirus (CMV)
Gammaherpesvirinae		Human herpes virus 6 (HHV6) Epstein-Barr virus (EBV) (very rare)

as the most frequently identified viral agent. In this study chronic heart failure (CHF) was a risk factor for virus infections.

## Viruses causing CAP

### *Adenovirus (Adv)*

The virus is composed of nonenveloped, icosahedral particles containing a double-stranded linear DNA genome. They are 70–90 nm in diameter and consist of 252 capsomers (hexons and pentons) with filamentous glycoproteins (fibers) on the penton bases, which display characteristic different lengths. Human adenoviruses have been classified into six species (subgen-

era) A-F on the basis of antigenicity and other biological properties (hemagglutination, tumorigenicity in animals). This is in quite good agreement with classification based on genomic differences, like DNA homology of less than 20% between viruses of the different subgenera. Serotype-specific epitopes are predominantly found on the hexon capsomere and the terminal part of the fiber, and are defined by the quantitative neutralization test. At present, 51 serotypes have been identified by neutralizing antibodies and nine of these are documented as respiratory pathogens. Adenoviruses are very resistant, also against proteolytic enzymes in the intestinal tract.

The species C adenoviruses (1, 2, 5 and 6) are endemic and are responsible for approximately 60% of all human adenovirus infections (Adv 6 only for 4%) and for more than 80% of the adenovirus infections (most commonly Adv 1 and 2) early in life, whereas they cause 15% of symptomatic lower respiratory tract infections. After primary infection, C viruses may be shed in feces for months or even years. Following the initial infection the C viruses establish a lifelong, asymptomatic, persistent infection, with currently unknown state of viral persistence. Probable places of persistence are tonsils and adenoids. New data suggest that human mucosal T-lymphocytes may harbor C adenoviruses in a latent form [8].

Premature infants are at high risk to develop disseminated neonatal adenovirus infection with pneumonia and high lethality [9]. In infants and children adenovirus infections primarily occur between 6 months and 5 years and are responsible for 4–10% of childhood pneumonias. Outbreaks in predominantly healthy children are most frequently associated with type 7, followed by types 3 and 21 [10–12]. For Adv 7-infected children at high risk the mortality rate is up to 40% and 12% in healthy children [13]. Adv differ in their ability to induce inflammatory response in lung tissue, but particularly Adv 7 is involved in severe lung inflammation and neutrophil infiltration [14].

The incidence of Adv infection may vary and depends on the detection assays and the study population. In general it is higher in children (21%) than in adults (9%) [15, 16]. In 50% of the described cases a viral coinfection was recognized [8, 17]. It is likely that Adv infections were caused also by reactivation of endogenous virus. Further, these infections are not linked to seasonal pattern.

Adenovirus epidemics do occur in human populations living in crowded conditions with poor hygiene. A large outbreak, predominantly caused by types 4 and 7, was observed between 1950 and 1960 in 10% of the recruits in the US military. Ninety percent of the Adv-infected persons developed pneumonia. Vaccination against adv 4 and 7 since 1971 has effectively reduced illness from these serotypes [18]. Recent epidemiological studies have shown that latent subgroup C viruses are involved in some chronic diseases in immunocompetent patients which are at high risk for pneumonia induced by other viral pathogens [19, 20].

Table 3. Examples of epidemiological data of adenovirus pneumonia

Patients no	Age / manifestation	Risk factor	Viral infections %	Pneumonia %	Adv sero-type	Study
7588	<5 years / ARD	mostly none	~ 40	0,4	?	[17]
1640	<5 years / LRTI	mostly yes immunodeficient	43–50	4–10	7, 3, 2	[13]
75	5–14 years / CAP	None	65	12	?	[21]

## Therapy and resistance

Disseminated adenovirus infections are difficult to treat and the agents used, ribavirin and cidofovir, yielded variable results. The benefit of intravenous ribavirin (a synthetic guanosine analog) treatment in life-threatening disease especially in immunocompromised patients, seemed to be better if antiviral therapy was started early. Other reports could not show a clear beneficial effect. *In vivo* as well as *in vitro* data suggest that susceptibility to ribavirin is highly dependent on the virus species [22, 23]. Viruses of subgroup C were shown to be sensitive to ribavirin, whereas serotypes of the subgroups A, B, D, E and F were resistant.

Despite a high level and long treatment in some patients no changes in sensitivity of the virus isolates against ribavirin to date have been reported.

Cidofovir is a nucleotide analogue of cytosine with an effective *in vitro* activity against different DNA viruses, adenoviruses included. Treatment is indicated for severe, disseminated Adv infections, but results are varying and treatment is limited by severe nephrotoxicity. Emergence of resistant Adv has been observed only in experimental systems.

## *Human coronavirus (HCoV)*

Coronaviruses are spherical, pleomorphic enveloped viruses with a diameter of 80–200 nm. They possess the largest genomes of all RNA viruses. The single-stranded positive RNA is associated with the nucleoprotein N forming the helical nucleoprotein complex. It is surrounded by the envelope, which contains three characteristic surface structures, the S protein, the membrane protein M and the envelope protein E. Oligomers of the S protein are formed to spikes on the virion surface and resemble a solar corona. The S protein determines cell tropism, is responsible for pathogenicity and is the strongest inducer of neutralizing antibodies. Some coro-

Table 4. Examples of epidemiological data of coronavirus pneumonia

Patients no	Age / manifestation	Risk factor	Viral infections %	Pneumonia %	HCoV	Study
501	<2 to >65 years ARTI	mostly none	37	0,4	-OC43	[25]
316	<65 years ARTI	CHF, COPD	~ 30	0,6	-OC43, -229E	[24]
418	>65 years CAP	cardio- pulmonary	?	2,4	-HKU1	[29]

naviruses contain a fourth envelope protein with a hemagglutinating and esterase activity. Four serogroups have been distinguished containing three human pathogens: group 1 with the prototype HCoV-229E, group 2 with the known HCoV-OC43. In 2003 the severe acute respiratory syndrome (SARS) led to the detection of a novel coronavirus. The consecutive extensive research in this field led to the discovery of further coronaviruses (see the following).

After rhinoviruses, coronaviruses are most frequently associated with the “common cold” (15–30%) in young adults, and they seem not to pose a risk for healthy elderly. Non SARS coronaviruses rarely cause pneumonia. However, they may cause diseases of the lower respiratory tract (LRT) in infants, immunocompromised patients, patients with underlying diseases and in frail older adults. During endemic outbreaks of HCoV-OC43 and -229E only elderly patients at risk rarely develop pneumonia [24, 25]. HCoV-NL63 (first described in 2004 in the Netherlands), but although widespread within the human population it is seldom responsible for pneumonia in children [26–28]. Recently, a novel coronavirus HCoV-HKU1 from an 71-year-old patient with COPD and pneumonia was described in Hong Kong [29]. HKU1-associated pneumonias so far described, occur from winter to spring, predominantly in the elderly (80% were >65 years) with comorbidity [30].

In November 2002, a new emerging disease, SARS was described in China as a contagious, potentially lethal atypical pneumonia. The SARS-CoV originated from animal viruses, which could be the result of recombination between mammalian and avian coronaviruses. By June 2003, worldwide 8447 cases of this illness from >30 countries were registered with more than 800 deaths (lethality 9.5%). SARS-CoV seems to have a distinct cell entry pathway. The initial infection is possible with an extreme low infectious dose resulting in the generation of proteases in the lung, which are responsible for a 100- to 1000-fold more efficient rate of infection [31]. In younger children SARS often induces a relatively mild and nonspecific respiratory illness [32, 33]. In a Chinese study the overall mortality rate was 19.7%, but increased to 78.6% in the patient group with serious underlying diseases [34].

## Therapy and resistance

There is no information about antiviral treatment of HCoV infections and the rare cases of HCoV associated pneumonia. At present also no standard antiviral therapy can be recommended for SARS. Ribavirin and corticosteroids used in severely ill patients seemed to be effective and a better outcome was reported after combination therapy with lopinavir/ritonavir, ribavirin plus steroid [35]. Nothing is known about emergence of resistant virus variants.

### *Human influenza virus / avian influenza virus*

Human and avian influenza viruses are described in separate chapters within this book. Only the epidemiological association with CAP and the virological diagnosis are discussed in this chapter.

Children (1 to 5 years) and adults with comorbidity, predominantly chronic heart disease and broncho-pulmonary dysplasia, pregnant women in the second or third trimester and persons >65 years are at high risk for influenza virus-associated pneumonia. Using sensitive methods it has been shown that the prevalence of influenza infections in older children with CAP may be higher, and mixed infections, mostly viral/bacterial infections were documented in up to 35% [3, 21]. Influenza A virus-associated pneumonia in pregnancy is accompanied by higher morbidity and lethality and infants may be born preterm with low weight. Although pneumonia in elderly patients may present only with few respiratory signs, recovery is prolonged, especially in the frail elderly, where the incidence of pneumonia is highest [7, 36]. Mixed infections are more common in the older age groups, but single virus infections are seen increasingly in the patients over 65 years [37]. Influenza B virus-associated pneumonia is rare and has been reported in single cases in children [38, 39].

Table 5. Examples of epidemiological data of influenza virus pneumonia

Patients no	Age / manifestation	Risk factor	Viral infections %	Pneumonia %	Study
514	CAP	10% premature 23% comorbidity			[40]
126	0–1 year		42	0,8	
241	1–<5 years		21	5,8	
147	5–16 years		6,8	1,4	
75	5–14 years CAP	none	65	6,7	[21]
338	>65 years CAP	CHF	18	11,5	[7]

### *Parainfluenza virus (HPIV)*

HPIV are pleomorphic enveloped viruses of between 150 and 300 nm. They contain a helical nucleocapsid with single-stranded negative RNA. It encodes at least six structural proteins and two nonstructural proteins. Important are the two envelope glycoproteins HN (hemagglutinin-neuraminidase) and F (fusion), responsible for neutralizing antibodies. Within the family of the paramyxoviruses only HPIV express neuraminidase activity. Four major HPIV serotypes exist which are divided into subtypes and genotypes with steadily occurring antigenic variations. Types 1, 2 and 3 are distributed worldwide, while type 4 is predominantly spread in America.

The four serotypes are distinct in their epidemiological and clinical behavior. HPIV 3 is endemic throughout the year, but there are yearly epidemic outbreaks from winter to spring. HPIV 1 and 2 are associated with a biennial epidemic pattern with the peak from fall to winter. Parainfluenza-viruses 1 to 3, particularly type 3, seem to have the highest virulence and the capacity to persist.

The majority of type 1 infections occur in children between the second and third year of life, whereas 60% of type 2 are in children younger than 5 years with a peak incidence between the first and second year. They are most commonly associated with croup or laryngitis, but may also cause pneumonia. Type 1 can be found in hospitalized, previously healthy adults and may be involved in bacterial pneumonias.

HPIV-3 infections occur in 40% of young infants in the first year of life and in most children in the first two years. Pneumonia with type 3 is seen primarily in the first 6 months of life, similar to RSV, but with lower frequency [41]. Like RSV HPIV can reinfect both children and adults with predominantly mild respiratory tract symptoms accompanied by low and short virus shedding [42, 43].

In childhood CAP parainfluenzavirus 1–3 infections beside RSV and influenzaviruses are the most common viral pathogens, which are involved in up to 10% of the diseases [3, 4, 40, 44]. Parainfluenza virus infections have been reported in the elderly with pneumonia in up to 12% of cases [43]. Viral CAP in adults with comorbidity like COPD or CHF is characterized by a more severe clinical outcome [7, 45]. Viral/bacterial coinfections (approximately 20%) are observed frequently.

### Therapy and resistance

Ribavirin has *in vitro* activity against HPIV and both aerosolized and intravenous ribavirin have been used. There are anecdotal reports about reduction of clinical signs and viral load in immunocompromised patients, when

treatment was started early after onset symptoms. Ribavirin resistant virus variants have not been described.

### *Respiratory syncytial virus (RSV)*

RSV is a negative-strand RNA virus. It is pleomorphic and has a size of 150–300 nm in diameter. The genome encodes eight structural and two nonstructural proteins. The helical capsid is surrounded by an envelope with three surface glycoproteins, the fusion (F) protein which mediates membrane fusion with the host cell resulting in viral penetration, the G protein which is responsible for attachment to the host cell, and an SH protein with unknown function. In contrast to other paramyxoviruses a hemagglutinin and neuraminidase function do not exist. The G protein has the highest degree of antigenic diversity in RSV. It accounts for the strain-specific epitopes and allows the classification into two antigenic groups RSV-A and RSV-B with multiple genotypes [46]. Immunologically important are the F and the G glycoproteins inducing protective neutralizing antibodies. However, immune-protection is not complete and early reinfections occur. The G glycoprotein is produced as a membrane bound and a secreted form. The secreted form is able to modulate the innate immune response to RSV and priming with this protein increases the severity of illness after RSV reinfection.

In children with severe disease caused by RSV the type 2 Th-cell response seems to be dominant. The G protein induces an unbalanced Th-cell response, which in the lung could result in airway hyperresponsiveness, mucus hypersecretion, and inflammation, and may contribute to peripheral blood and pulmonary eosinophilia [47].

RSV infections occur worldwide peaking in the winter months in temperate climates and in the rainy season in tropical climates. A and B viruses and their multiple variants generally circulate simultaneously within epidemical outbreaks. Yearly outbreaks are possible, because the pattern of the circulating RSV strains changes, depending on the local strain-specific immunity in the human population [48].

RSV is the most important cause of acute respiratory tract viral infection in infants. Primary infections are symptomatic with a spectrum of clinical manifestations from mild upper tract illness to life-threatening pneumonia. RSV accounts for 50% of all cases of pneumonia during the first 2 years of life. The peak incidence of RSV lower respiratory tract infection (LRTI) is between 1 and 6 months of age. Maternal RSV-specific antibodies rapidly decrease after birth to approximately 6% at 3 months. This may be the reason for the high frequency of RSV infections before 3 months of age [49]. Premature infants (28 to 32 weeks) are at risk for 12 to 6 months after birth. All children get infected once until the

age of 2 years, but 50% of them already had experienced re-infections [4, 21, 40, 50–52].

There are numerous independent risk factors for severe RSV infection including genetic factors that are under discussion. In immunocompromised children, RSV-related mortality (with very high RSV load) was 15%, and for children with primary immunodeficiencies 40% [53].

In immunocompetent adults younger than 60 years RSV reinfections are generally mild and may contribute to 2–4% of the lower respiratory tract infections. In elderly people reinfections can induce life-threatening pneumonia. Similar to children there may exist specific risk factors for severe RSV disease. In different studies in adult and elderly patients 3–10% developed RSV diseases depending on risk factors. The infections accounted for 11% of hospitalizations for pneumonia [54–56].

The development of a prophylactic vaccine against RSV was pursued with a high priority. The formalin-inactivated RSV vaccine developed in the 1960s unfortunately led to a more severe lung disease in vaccinated children after a subsequent RSV challenge [57].

## Therapy and resistance

Ribavirin is licensed to treat severe RSV-associated diseases in children. Immune globulin for intravenous administration and a humanized monoclonal antibody preparation (palivizumab (Synagis)) are designed to prevent or reduce the severity of RSV infection. It has been shown that in RSV-infected infants with lower respiratory tract disease ribavirin aerosol therapy improves clinical outcome, but may be effective in adults as well. Despite high dose and prolonged treatment in some patients no ribavirin-resistant RSV variants have been isolated [58].

## *Human metapneumovirus (HMPV)*

This recently identified paramyxovirus is most closely related to the pneumovirus RSV, but HMPV differs from RSV in two aspects: it is lacking the non-structural proteins (NS1 and NS2) and it has a different gene constellation. HPMV is classified into two main lineages, A and B, based on sequence analyses of the F gen. Further sequence analysis of different HMPV genes including the G gene are required for refined characterization of the virus isolates. The two major groups with numerous genotypes cocirculate throughout the year. Sometimes even genetically distinct strains of HMPV are circulating during the same year. Most infections have been detected during late winter and early spring following the peak activity of both RSV and influenza virus.

The virus was first detected in young children in 2001 in the Netherlands [59], but serological studies showed that the virus has been circulating in

Table 6. Epidemiological data of metapneumovirus pneumonia

Patients no	Age / manifestation	Risk factor	Viral infections %	Pneumonia %	Study
90	0–1 years ARD	none	47	10	[61]
208	<3 years ARD	10%	78	1	[62]
145	adults CAP+EA	COPD / CHF / asthma	19	2,8	[64]

humans for at least 50 years. HMPV has a worldwide distribution and association with respiratory illness in all age groups. It causes upper respiratory tract infections, but is also associated with lower respiratory tract infections. It has been suggested that most severe HMPV infections occur in children <2 years of age and seem to peak in the third and fifth months of life, somewhat later than RSV. It is found less frequently in hospitalized children than RSV and the clinical course may be milder. Based on the presence of HMPV antibodies approximately 55% of children at the age of two and 100% at the age of 5–10 years had a HMPV history. HMPV like RSV may cause clinically important reinfections in late childhood and adult life, but the highest infection rate was found in young adults.

HMPV also can be responsible for pneumonia in premature born babies [60] and other persons at risk. Although in the study of Maggi et al. [61] the number of infants with age less than 2 years is small, the majority of the HMPV-infected children developed pneumonia. The incidence in this study was higher (33%) than in other reports, but the difference is related to the difference in the population of children studied [62, 63]. The rate of bronchopneumonias was higher in children with isolated HMPV infection than in children with mixed infections. Surprising in this study [61] was the detection of HMPV RNA in plasma of 41% of HMPV-infected children, like was shown also for RSV infections.

HMPV pneumonia clinically cannot be distinguished from RSV and influenza, but the disease seems to be somewhat less severe and there is a greater percentage of cases with underlying diseases (25%) compared to the influenza- or RSV-associated pneumonia (<10%). Small studies demonstrated that HMPV is a relatively important viral pathogen, which can also lead to pneumonia (14%), especially in elderly and adults with underlying disease like COPD, CHF, or asthma [64].

Additional studies will be needed, especially year-long active surveillance over consecutive years with analysis of more data from future respiratory seasons to fully define the clinical and epidemic impact of HMPV infections.

## Therapy and resistance

No antiviral agents or antibody preparations are currently available for the treatment of HMPV infection. It was shown that ribavirin and a polyclonal intravenous immunoglobulin had equivalent *in vitro* activity against both HMPV and RSV. Treatment may be considered for severe HMPV infection in immunocompromised patients [65]. No reports about resistant HMPV exist.

### *Picornaviruses (human rhinovirus (HRV) / human enterovirus (HEV))*

Picornaviruses are nonenveloped particles and with a diameter of 30 nm they are very small. They consist of a single positive-strand RNA genome surrounded by an icosahedral capsid composed of 60 protomers. Each protomer consists of three nonglycosylated surface proteins (VP1 to VP3) and internal proteins (VP4). In the center of each protomeric unit is a canyon where antigenic sites and structures can be found binding to receptors of the target cells. There are over 100 immunologically distinct rhinovirus serotypes and two species A and B can be distinguished. Rhinoviruses are acidlabile and differ in membrane receptor recognition. Further, more than 60 enterovirus serotypes exist.

Rhinoviruses are ubiquitous and infections by relatively low infectious doses occur throughout the year. They are the most important pathogens of the “common cold,” in 80–90% of humans during the peak season in late autumn. Rhinoviruses are able to infect the lower respiratory tract. It has been shown in experimental infections that a lower respiratory infection with rhinoviruses (detected in bronchial biopsy samples) during “common cold” is not unusual.

Picornaviruses are the most frequently detected virus in respiratory tract infections in the first year of life. In hospitalized infants with acute expiratory wheezing illness respiratory picornaviruses (rhino- and enteroviruses) are found in 42% of the cases. In older children picornaviruses are predominant with 65% at the age of 1–2 years and with 82% in children older than 3 years [66, 67].

Earlier studies have not included rhinoviruses, but current data suggest that they are rarely involved in pneumonia in infants, young children and in older adults. Rhinoviruses can cause pneumonia in children of the age group 0–6 months, but the highest isolation rate was found in children between 6 and 12 months with the same frequency as RSV [4, 24, 68, 69]. Coinfections of rhinoviruses and bacteria were found in pediatric patients with CAP in about 10%. The inflammatory response to rhinovirus infection is strong and several cytokines are related to pneumonia like IL-6, which may be an important factor in rhinovirus pathogenesis [68, 70]. In

Table 7. Examples of epidemiological data of picornavirus pneumonia

Patients no	Age / manifestation	Risk factor	Viral infections %	Pneumonia %	Study
502	ARTI	56% passive smoking	43	3	[69]
178	0–6 months		46	2,8	
184	6–12 months		40	4,9	
96	>12 months		38	1	
254	0,1–17 years CAP	?	62	23	[4]
316	>65 years ARTI	CHF, COPD	30	0,5	[24]

a recent review about enterovirus-associated respiratory tract diseases Rotbart et al. [71] reported, that 13% of infected patients presented with pneumonia. In contrast, Kellner et al. [69] in a prospective study recovered enteroviruses only sporadically from children with upper respiratory tract disease. However, case reports exist about fatal pneumonias in congenital and neonatal echovirus-infected infants. In these rare and sporadic cases a maternal disease has been reported in 59 to 68% [71–75]. Epidemic outbreaks of hand, foot, and mouth disease with enterovirus 70/71 in children have been observed, where after CNS involvement pulmonary edema appeared [76].

Using modern diagnostic methods it is increasingly recognized, that rhinovirus and enterovirus infections are the most common reasons for unnecessary antibiotic therapy.

## Therapy and resistance

The most promising antiviral of the so called WIN compounds is pleconaril with a broad potent anti-EV and anti-RV activity. It binds to hydrophobic sites in the base of the capsid canyons and inhibits uncoating of the capsid in all enteroviruses. Rhinoviruses of the species B, have a significant reduced susceptibility to pleconaril. For therapeutic application it will be important to differentiate between natural occurring resistance to pleconaril in B rhinoviruses and the emergence of RV resistance under pleconaril treatment [77].

Pleconaril has been shown to be effective in experimental studies and to significantly reduce clinical symptoms in treated adult volunteers. It is available for life threatening enterovirus infections in immunocompromised patients. Ten percent of the enterovirus isolates have been shown to be resistant to pleconaril and some patients did not respond (resistant virus has been identified). Rupintrivir is an inhibitor of the virally encoded 3C

protease, which is still under investigation for treatment of rhinovirus infection in immunocompetent patients [78].

### *Hantavirus (HV)*

Hantaviruses are enveloped, predominantly negative-strand RNA viruses (ambisense). The genome of these viruses consists of three different single-stranded RNA segments. The segments L, M and S encode the viral polymerase, a glycoprotein processed into G1 and G2 glycoproteins located in the envelope, and a nucleocapsidprotein. Virus particles are of spherical shape with a diameter of 80–120 nm, but also elongated forms are seen (170 nm).

Hantaviruses are important zoonotic pathogens, primarily of rodents. The infections are persistent and most of them seem to be asymptomatic in the natural rodent hosts. Antibodies against hantaviruses are also present in nonnatural hosts, other wild and domestic animals. Beside the transmission to humans through human/rodent contacts, infections are acquired by inhalation of virus-contaminated aerosols of rodent excreta (saliva, urine or feces) [79]. An occasional transmission may occur from person to person as was documented for Andes virus in Argentina.

Some hantaviruses belong to the so called emerging pathogens. They cause an influenza-like acute pulmonary disease in North, Middle and South America, the hantavirus pulmonary syndrome (HPS). The first HPS was recognized in 1993 in the USA and was caused by a number of hantavirus variants, e.g. Sin Nombre virus (SNV). Symptoms of HPS may vary, depending on the virus genotype. In 1995, cases of HPS were reported in South America and a new strain – the Andes hantavirus – was identified. In late 1999 and early 2000 first outbreaks through an again novel hantavirus, the chocho virus, were documented in Central America. The overall mortality was about 44%, but is declining as a consequence of better recognizing less severe forms of the infections and a better medical management. Confirmed cases of HPS so far include children, but in the majority of cases adults (19 to 58 years). SNV accounts for a small number of pediatric cases in the US, whereas Andes virus was found in pediatric patients (Chile, South Argentina) in a higher proportion (16%) [80–82].

The incubation period for Andes virus in Chile was 5 to 25 days. Febrile prodromi last for approximately 4 days followed by a rapid progression to moderate to severe respiratory distress.

### Therapy and resistance

For hantavirus diseases no established specific therapy is currently available. Ribavirin shows activity against HV, but this has not been well documented in studies. Emergence of resistant HV is unknown.

## Nonconventional respiratory viruses

### *Measles virus*

In developing countries the frequent complications of measles virus infection are responsible for the mortality rate. Measles virus infection causes a transient and strong immunosuppression, which is the reason for the increased susceptibility to other viral or bacterial infections. In 3–4% of the infected patients measles virus can cause pneumonia, which can be present either as primary measles virus pneumonia or as atypical measles virus pneumonia, but most patients develop secondary bacterial pneumonia. Measles pneumonia rarely occurs in young adults as shown in 1976 to 1979, when a measles outbreak in US Air Force recruits was responsible for 106 cases with pneumonia (3.3%), of which two-thirds had a secondary bacterial infection [83]. During pregnancy measles virus infections induce a higher number of pneumonias in the mother [84, 85]. There are a few reports about preterm and newborn infants in which measles pneumonia was observed [86].

### *Herpes viruses*

After primary infection herpes viruses are able to persist lifelong in their human host, typically as latent infection, but they also reactivate under immunosuppression and then some of them, like CMV, HSV, VZV, EBV and HHV6, may induce pneumonia.

HSV-1 pneumonia may occur occasionally in high-risk persons. Rarely disseminated HSV infections during pregnancy have been reported, as in the case of a previously healthy woman with a fatal progressive HSV-2 pneumonia in the third trimester of pregnancy [87]. Ramsey et al. reported 20 patients with pneumonia, in which they could differentiate between cases with focal HSV pneumonia as a result of HSV spreading to the lung parenchyma and cases with interstitial pneumonia as a result of a hematogenous dissemination of HSV [88].

In immunocompetent adults primary VZV infection is uncommon, but the incidence is increasing (5–10%) and VZV pneumonia is the main complication (incidence 5.5%–16.5%) with high mortality. Some patients may develop secondary bacterial pneumonia. Most patients (76.7%) do have at least one known risk factor like pregnancy, smoking or chronic obstructive pulmonary disease [84, 89–91]. VZV beside influenza virus is the most common pathogen, causing pneumonia during pregnancy, predominantly in the second and third trimester. The risk of primary VZV infection for VZV pneumonia during pregnancy (0.1–18.3%) is higher if patients are smokers or manifest multiple (>100) skin lesions. The mortality rate before a possible antiviral intervention was significantly higher in pregnancy (41% *versus*

1.5–12.1%) [89, 92, 93]. VZV pneumonias as consequence of a primary VZV infection in childhood are rare [94].

CMV-infected preterm infants can develop a chronic lung disease, which may be associated with CMV pneumonia with high mortality. Ganciclovir treatment has been shown to rapidly improve symptoms, a fact that supports CMV causality of pneumonia [95–97]. Extremely rare are reports about CMV pneumonia in previously healthy adults [98]. In patients with lymphoma CMV pneumonia is less common and the incidence after chemotherapy and corticosteroid application is approximately 1% with a mortality rate of 30% [99].

Two genetically distinct variants HHV-6A and -6B do exist, but in primary infection the variant -6B is dominant. During primary infection a severe respiratory disease is an extremely rare complication. In a case report from Knox et al. [100] a fatal pneumonitis due to HHV-6 infection is documented in an infant with severe T lymphocytopenia. Whereas for immunocompromised patients (BMT) HHV-6 pneumonia is documented, in immunocompetent patients an etiologic role of the reactivated HHV-6 infection in pneumonia is not clearly defined [101, 102]. Another report of an extremely rare case with HHV-6-associated pneumonia is documented by Merk et al. [103], where an apparently immunocompetent young woman developed a fatal pneumonia.

A mild asymptomatic pneumonitis is described in 5–10% of the cases with infectious mononucleosis, but a severe pneumonitis as result of primary EBV infection is rare in immunocompetent patients [84, 104, 105]. In patients with lymphocytic interstitial pneumonia EBV DNA has been found in lung tissues of infants, children and of adults as well [106–108]. In autopsy cases with diffuse interstitial pneumonia EBV DNA could be detected in leukocytes and pneumocytes and frequently in airway epithelial cells [109]. The EBV-associated lymphocytic interstitial pneumonia without HIV infection has been reported predominantly in adults. This is also true for a rare chronic interstitial lung disease due to EBV, mainly seen in adults, but also shown in two infants in early months of life [106, 110].

## **Diagnosis of viral pulmonary infection**

Especially in adult patients respiratory virus infections are extremely underdiagnosed since adequate and possible virological diagnosis is not routinely performed.

For diagnosis of acute respiratory infections the detection of infectious viruses or viral components (proteins, nucleic acid) is the method of choice, whereas antibody detection is not relevant and should only be used in selected patients at later timepoints after infection using paired sera.

## *Specimen*

*Nasopharyngeal aspirate (NPA)* is the gold standard for the detection of all major respiratory viruses, predominantly adopted in infants and children. NPA is taken by suction of cell-containing mucosal secretion from the nasopharyngeal area. Approximately 0.5 ml fluid should be collected into 2 ml of viral transport medium.

*Nasal lavage* can be obtained with less discomfort for the patient by gently instilling 2 ml PBS at room temperature into each nostril and by simultaneously suctioning into a sterile trap; the sensitivity for virus detection is comparable to that of NPA, but is controversially reported for RSV [111, 112].

*Nasopharyngeal swab*, taken usually from adults, is obtained by deep bilateral nasal and posterior pharynx swabs with sterile cotton swabs, which should then be placed in 3 ml viral transport medium (VTM) [113]. Calcium alginate swabs or swabs with wooden sticks may contain substances which inactivate some viruses and inhibit PCR testing and should not be used!

*Tracheal aspirate* can be collected from intubated patients after instillation of 4–10 ml sterile normal saline into the endotracheal tube and by suctioning into a sterile trap; this is the most easily obtained specimen from lower respiratory tract in these patients [114].

*Induced sputum* should be collected in the morning after rinsing mouth and throat with sterile hypertonic saline. Thereafter sputum has to be collected in a sterile container. Induced sputum represents a more complete sampling of the respiratory tract and is comparable with nasopharyngeal aspirate specimens. While induced sputum might be used for virus detection from the lower respiratory tract, the obligate admixture of saliva with the accompanied flora is an obvious disadvantage. Nevertheless, with minimal saliva contamination it is a valuable alternative material to BAL [115, 116].

*Bronchioalveolar lavage (BAL)* has a high diagnostic yield for infectious pathogens predominantly in immunocompromised patients with lung infiltrates.

*Transbronchial biopsy (TBB) / lung biopsy*. This invasive technique can in addition to BAL significantly improve the diagnostic yield [117].

*EDTA blood* in immunosuppressed patients may facilitate diagnosis of some viruses (e.g. adenovirus, CMV) and disseminated virus infections and determination of the virus load may be predictive for disease and outcome; furthermore, virus load is important for monitoring of therapy.

## **Methods for detection of viruses**

### *Direct virus detection (rapid antigen detection / nucleic acid assay)*

Enzyme/immunoassays (EIA) for rapid viral antigen detection in airway secretions exist for almost all major respiratory viruses, but have been often

evaluated only for children and may vary in sensitivity and specificity (range between 60 and 95%). Results from EIAs for detection of single respiratory viruses are usually available between 10 min to 3 h. Alternatively a screening for respiratory viruses in an airway secretion can be achieved by indirect immunofluorescent antigen assay (IFA) (sensitivity 85–95%; specificity 95–99%) using a pool of monoclonal antibodies, whose specificity is directed against RSV, influenzaviruses A, B, parainfluenzaviruses 1, 2, 3 and adenovirus. The examination of several cell spots of a cytospin preparation on slides with pooled and single antisera allows viral antigen detection and typing within 2 h. The results are strongly dependent on the quality of the clinical material. For elderly hospitalized and immunocompromised patients with virus associated LRTI the rapid virusantigen detection is insensitive.

When rapid conventional methods are not available, like for coronaviruses, metapneumoviruses, rhinoviruses or enteroviruses, the PCR has to be established.

### *PCR*

The PCR may be used alternatively to the mentioned antigen detection assays. It offers a great sensitivity and can be used for a wider range of viral pathogens. In principle PCR can detect and differentiate several viruses simultaneously in a single reaction mixture as multiple RT-PCR (multiplex PCR), but it provides a lower sensitivity than PCR for single pathogens and is more difficult to establish. For early detection and monitoring of viral infections the quantitative PCR represents a sensitive technique that delivers results within 3 h.

For diagnosis of disseminated virus infections with pulmonary manifestations frequently caused by adenoviruses, RSV in infants or nonconventional respiratory viruses, like CMV additional analysis of plasma or EDTA blood is helpful.

### *Detection of infectivity (short term culture / isolation)*

Clinical specimen namely secretions of the respiratory tract can be used for an infectivity assay in centrifuge enhanced shell vial culture (SVC). After inoculation of airway specimens on different cell systems, which are susceptible for many of the respiratory viruses, cell cultures are evaluated for early virus infection after 24–72 h with a pool of virus-specific monoclonal antibodies and afterwards for typing with the respective individual monoclonal antibodies by IFA.

Once isolated, the virus must be typed again preferentially with IFA using virus-specific monoclonal antibodies or otherwise increasingly by PCR.

Conventional cell culture with virus isolation has no impact on clinical decision and management of patients during hospitalization. Virus isolation is useful for a characterization of virus strains involved in epidemic outbreaks.

### *Antibody detection*

Serology is not useful during the acute phase of infection. Due to the delayed onset of the antibody response, approximately at the end of the first week of disease serological methods can detect antibodies. To use only serological methods for diagnosis of viral infections in CAP is not at all sufficient. Paired serum samples may be used to detect seroconversion or a fourfold increase in antibody titer comparing the first and the second serum.

Serology in addition is of limited value in newborns with maternal antibodies, in immunocompromised patients, in the elderly and patients receiving blood products. Antibody detection can be done with the complement fixation test (CFT, increasingly obsolete), ELISA-IgG, -IgA, -IgM, immunofluorescent assays, immunoblot and neutralization test.

## **Diagnostic comments on specific viruses**

### *Adenovirus*

Viruses can be detected in 25 to 72% of patients with disseminated infection in peripheral blood for more than 3 weeks. Detection of virus from multiple sites correlates with more severe disease and higher mortality. It is essential to monitor viral load during antiviral therapy. Phenotyping of isolates can be done by neutralization test, by hemagglutination test or by serotype-specific monoclonal antibodies and finally by PCR.

Group-specific antigens are used in CFT for antibody detection in the second week of disease. CFT is insufficient to detect antibodies in infants. IgG antibodies are detectable by ELISA at the end of the second week, whereas IgM antibodies are not detectable in all cases with primary infection [10].

### *Coronavirus*

HCoV antigen can be detected by IFT using rabbit antisera (no monoclonals available). Coronaviruses RNA can be detected with RT-PCR also in stool. SARS-CoV can be detected in plasma, but in urine and stool longer than 4 weeks. Virus load is unusually low in the early phase of SARS. A

chip-based test detecting 10 respiratory viral pathogens including coronaviruses has been developed. Non-SARS human coronaviruses are difficult to isolate. With SARS-HCoV no IgG and IgM antibodies can be found within the first 7 days of disease, seroconversion may be delayed up to 8 weeks and IgG does not persist [34, 118, 119].

### *Influenzavirus*

Rapid antigen detection tests are available (15 min) which differentiate between A and B viruses and some now include avian influenza virus H5N1. Sensitivity and specificity range from 60–95% and 52–99% respectively. RT-PCR is much more sensitive and allows virus typing. Virus isolation is still important for characterization of circulating influenza strains.

Antibody detection with ELISA-IgG, -IgA and IFA-IgG, -IgA is possible, but shows low levels of both IgG and IgA, a delayed peak antibody titer and shorter persistence of antibodies in elderly patients.

### *Parainfluenzavirus and respiratory syncytial virus*

In adults and elderly people virus shedding is lower and shorter. RT-PCR is more sensitive in patients with parainfluenzavirus and RSV infections, particularly reinfections, and it might be useful especially for rapid diagnosis in elderly.

For rapid RSV antigen detection in pediatric patients different antigen detection assays (20 min) with sensitivities between 61 to 92% and specificities between 93 to 98% are available [120]. Alternatively the rapid immunofluorescent antigen assay can be used.

Since RSV is thermolabile, it is important to use a qualified transport medium, and to have a cooled and short transport until processing (30 min). The shell vial culture assay shows the highest sensitivity (94.3%) and specificity (96.9%) for detecting RSV from NPA in children younger than 1 year.

Only 41% of RSV-infected children could be identified by serology and antibodies titers that develop are usually low, although both serum and secretory antibodies are produced [121].

### *Human metapneumovirus*

Commercial monoclonal antibodies are available for IFA. The sensitivity and specificity of this test is 73.3 and 97% respectively and results agree with RT-PCR in 89.6% [122].

Isolation of HMPV is difficult and regardless of CPE the use of RT-PCR to enhance the HMPV identification in cell culture is indicated.

No commercial tests are available for antibody detection.

### *Picornaviruses (rhinovirus / enterovirus)*

Due to more than 100 distinct serotypes a rapid detection of rhinovirus capsid antigen cannot be developed. The application of molecular assays has markedly increased the detection rate of picornaviruses in acute respiratory infections. Rhinovirus culture is the “gold standard,” but it takes 3 to 7 days. The sensitivity of PCR is superior to the infectivity detection in the cell culture.

No reliable typespecific serological test for rhinovirus infections exists. Enterovirus serology by neutralization tests is possible but difficult and not completely reliable.

### *Hantavirus*

Currently no antigen detection assay is available. RT-PCR for hantavirus RNA detection is useful to detect infection and to identify the viral genotype. RT-PCR for viral RNA detection can be done from whole blood or serum during the acute phase in the first 10 days of illness.

Virus isolation is inefficient and there is no routine assay.

Serum specimens were tested for IgM and IgG antibodies by ELISA using Sin Nombre virus antigen, according to the guidelines of the US Centers for Disease Control (CDC). Positive results indicate infections with new world hantaviruses. IgM antibodies can be detected in all acute cases, maximal IgG level occurs during the first week of illness and are detectable after disease for a relatively long period.

### *HSV, VZV and CMV*

Since HSV, VZV and CMV pneumonia can be effectively treated it is important to rapidly investigate specimens from the lower respiratory tract, because pneumonia can only be established on the basis of BAL or lung tissue examined by PCR (HSV, VZV) and/or shell vial culture (HSV). Serologic tests are not relevant in case of HSV reactivation.

Primary VZV infection can be confirmed by IgM detection and by IgG seroconversion.

In premature infants with risk for CMV pneumonia CMV DNA can be found directly by PCR in urine and in the pharynx. Virus detection in air-

way specimens (virus load in BAL) and lung tissue will be positive before seroconversion.

## Summary

In various studies and case reports it has been shown that respiratory viral pathogens are frequently involved in community-acquired pneumonia. Age groups at risk are the very young and elderly, as well as persons with comorbidity, where immune response is restricted.

The frequency of detection of respiratory viruses varies in different studies due to methodological problems, patient selection and the fact that only detection of specific viruses was performed. With modern diagnostic tools it has been increasingly realized that rhinovirus and enterovirus infections are the most common reasons for unnecessary antibiotic therapy, often also adenovirus infections. Future studies have to consider the whole range of viral agents that can be involved in community-acquired pneumonia.

In recent decades several new respiratory viruses have been described and it is reasonable to assume that new viruses or virus types from different virus families will emerge in the future, often after having crossed a host species barrier with high pathogenic potential for severe pulmonary diseases.

Most of the relevant infections can be diagnosed by virus detection.

Several studies in children have documented a significant proportion (25 to 30%) of viral/bacterial infections beside isolated virus infections. Children with isolated virus infection tended to be younger than coinfecting children. The implications of coinfecting agents in epidemiology, pathogenicity and clinical outcome have to be elucidated.

## References

- 1 Welte T, Marre R, Suttorp N (2004) Competence network "community acquired pneumonia" (CAPNETZ). A first interim report. *Internist (Berl)* 393–401
- 2 Shah PB, Giudice JC, Griesback R Jr, Morley TF, Vasoya A (2004) The newer guide-lines for the management of community-acquired pneumonia. *J Am Osteopath Assoc* 104: 521–526
- 3 Michelow IC, Olsen K, Lozano J, Rollins NK, Duffy LB, Ziegler T, Kauppila J, Leinonen M, McCracken GH, Jr (2004) Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 113: 701–707
- 4 Juven T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, Eskola J, Saikku P, Ruuskanen O (2000) Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 19: 293–298
- 5 Juven T, Mertsola J, Waris M, Leinonen M, Ruuskanen O (2004) Clinical

- response to antibiotic therapy for community-acquired pneumonia. *Eur J Pediatr* 163: 140–144
- 6 Virkki R, Juven T, Mertsola J, Ruuskanen O (2005) Radiographic follow-up of pneumonia in children. *Pediatr Pulmonol* 40: 223–227
  - 7 de Roux A., Marcos MA, Garcia E, Mensa J, Ewig S, Lode H, Torres A (2004) Viral community-acquired pneumonia in nonimmunocompromised adults. *Chest* 125: 1343–1351
  - 8 Garnett CT, Erdman D, Xu W, Gooding LR (2002) Prevalence and quantitation of species C adenovirus DNA in human mucosal lymphocytes. *J Virol* 76: 10608–10616
  - 9 Rieger-Fackeldey E, Aumeier S, Genzel-Boroviczeny O (2000) Disseminated adenovirus infection in two premature infants. *Infection* 28: 237–239
  - 10 Aberle SW, Aberle JH, Steininger C, Matthes-Martin S, Pracher E, Popow-Kraupp T (2003) Adenovirus DNA in serum of children hospitalized due to an acute respiratory adenovirus infection. *J Infect Dis* 187: 311–314
  - 11 Carballal G, Videla C, Misirlian A, Requeijo PV, Aguilar MC (2002) Adenovirus type 7 associated with severe and fatal acute lower respiratory infections in Argentine children. *BMC Pediatr* 2: 6
  - 12 Chuang YY, Chiu CH, Wong KS, Huang JG, Huang YC, Chang LY, Lin TY (2003) Severe adenovirus infection in children. *J Microbiol Immunol Infect* 36: 37–40
  - 13 Hong JY, Lee HJ, Piedra PA, Choi EH, Park KH, Koh YY, Kim WS (2001) Lower respiratory tract infections due to adenovirus in hospitalized Korean children: epidemiology, clinical features, and prognosis. *Clin Infect Dis* 32: 1423–1429
  - 14 Booth JL, Coggeshall KM, Gordon BE, Metcalf JP (2004) Adenovirus type 7 induces interleukin-8 in a lung slice model and requires activation of Erk. *J Virol* 78: 4156–4164
  - 15 Leruez-Ville M, Minard V, Lacaille F, Buzyn A, Abachin E, Blanche S, Freymuth F, Rouzioux C (2004) Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. *Clin Infect Dis* 38: 45–52
  - 16 Howard DS, Phillips II GL, Reece DE, Munn RK, Henslee-Downey J, Pittard M, Barker M, Pomeroy C (1999) Adenovirus infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 29: 1494–1501
  - 17 Rocholl C, Gerber K, Daly J, Pavia AT, Byington CL (2004) Adenoviral infections in children: the impact of rapid diagnosis. *Pediatrics* 113: e51–e56
  - 18 Gray GC, Goswami PR, Malasig MD, Hawksworth AW, Trump DH, Ryan MA, Schnurr DP (2000) Adult adenovirus infections: loss of orphaned vaccines precipitates military respiratory disease epidemics. *Clin Infect Dis* 31: 663–670
  - 19 Hayashi S (2002) Latent adenovirus infection in COPD. *Chest* 121: 183S–187S
  - 20 Fujii T, Hogg JC, Keicho N, Vincent R, Van Eeden SF, Hayashi S (2003) Adenoviral E1A modulates inflammatory mediator expression by lung epithelial cells exposed to PM10. *Am J Physiol Lung Cell Mol Physiol* 284: L290–L297
  - 21 Tsolia MN, Psarras S, Bossios A, Audi H, Paldanius M, Gourgiotis D, Kallergi K, Kafetzis DA, Constantopoulos A, Papadopoulos NG (2004) Etiology of com-

- munity-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 39: 681–686
- 22 Morfin F, Dupuis-Girod S, Mundweiler S, Falcon D, Carrington D, Sedlacek P, Bierings M, Cetkovsky P, Kroes AC, van Tol MJ, Thouvenot D (2005) *In vitro* susceptibility of adenovirus to antiviral drugs is species-dependent. *Antivir Ther* 10: 225–229
  - 23 Lankester AC, Heemskerck B, Claas EC, Schilham MW, Beersma MF, Bredius RG, van Tol MJ, Kroes AC (2004) Effect of ribavirin on the plasma viral DNA load in patients with disseminating adenovirus infection. *Clin Infect Dis* 38: 1521–1525
  - 24 Falsey AR, Walsh EE, Hayden FG (2002) Rhinovirus and coronavirus infection-associated hospitalizations among older adults. *J Infect Dis* 185: 1338–1341
  - 25 Vabret A, Mourez T, Gouarin S, Petitjean J, Freymuth F (2003) An outbreak of coronavirus OC43 respiratory infection in Normandy, France. *Clin Infect Dis* 36: 985–989
  - 26 Vabret A, Mourez T, Dina J, van der HL, Gouarin S, Petitjean J, Brouard J, Freymuth F (2005) Human coronavirus NL63, France. *Emerg Infect Dis* 11: 1225–1229
  - 27 Moes E, Vijgen L, Keyaerts E, Zlateva K, Li S, Maes P, Pyrc K, Berkhout B, van der HL, Van RM (2005) A novel pancoronavirus RT-PCR assay: frequent detection of human coronavirus NL63 in children hospitalized with respiratory tract infections in Belgium. *BMC Infect Dis* 5: 6
  - 28 van der HL, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B (2004) Identification of a new human coronavirus. *Nat Med* 10: 368–373
  - 29 Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, Wong BH, Poon RW, Cai JJ, Luk WK, Poon LL, Wong SS, Guan Y, Peiris JS, Yuen KY (2005) Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 79: 884–895
  - 30 Woo PC, Lau SK, Tsoi HW, Huang Y, Poon RW, Chu CM, Lee RA, Luk WK, Wong GK, Wong BH, Cheng VC, Tang BS, Wu AK, Yung RW, Chen H, Guan Y, Chan KH, Yuen KY (2005) Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. *J Infect Dis* 192: 1898–1907
  - 31 Matsuyama S, Ujike M, Morikawa S, Tashiro M, Taguchi F (2005) Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. *Proc Natl Acad Sci USA* 102: 12543–12547
  - 32 Bitnun A, Allen U, Heurter H, King SM, Opavsky MA, Ford-Jones EL, Matlow A, Kitai I, Tellier R, Richardson S, Manson D, Babyn P, Read S (2003) Children hospitalized with severe acute respiratory syndrome-related illness in Toronto. *Pediatrics* 112: e261
  - 33 Cheng FW, Ng PC, Chiu WK, Chu WC, Li AM, Lo KL, Hon EK, Nelson EA, Leung TF, Ng WH, Wong E, Ip P, Fok TF (2005) A case-control study of SARS versus community-acquired pneumonia. *Arch Dis Child* 90: 747–749
  - 34 Mo HY, Xu J, Ren XL, Zeng GQ, Tan YX, Chen RC, Chan-Yeung M, Zhong NS (2005) Evaluation by indirect immunofluorescent assay and enzyme linked immunosorbent assay of the dynamic changes of serum antibody responses

- against severe acute respiratory syndrome coronavirus. *Chin Med J (Engl)* 118: 446–450
- 35 Cheng VC, Tang BS, Wu AK, Chu CM, Yuen KY (2004) Medical treatment of viral pneumonia including SARS in immunocompetent adult. *J Infect* 49: 262–273
- 36 Marrie TJ (2000) Community-acquired pneumonia in the elderly. *Clin Infect Dis* 31: 1066–1078
- 37 van Gageldonk-Lafeber AB, Heijnen ML, Bartelds AI, Peters MF, van der Plas SM, Wilbrink B (2005) A case-control study of acute respiratory tract infection in general practice patients in The Netherlands. *Clin Infect Dis* 41: 490–497
- 38 Lu KC, Chen PY, Huang FL, Yu HW, Kao CH, Lau YJ (2004) Influenza B virus associated pneumonia: report of one case. *Acta Paediatr Taiwan* 45: 242–245
- 39 van den Dungen FA, van Furth AM, Fetter WP, Zaijjer HL, van Elburg RM (2001) Fatal case of influenza B virus pneumonia in a preterm neonate. *Pediatr Infect Dis J* 20: 82–84
- 40 Weigl JA, Puppe W, Belke O, Neussuss J, Bagci F, Schmitt HJ (2005) Population-based incidence of severe pneumonia in children in Kiel, Germany. *Klin Padiatr* 217: 211–219
- 41 Hall CB (2001) Respiratory syncytial virus and parainfluenzavirus. *N Engl J Med* 344: 1917–1928
- 42 Henrickson KJ (2003) Parainfluenzaviruses. *Clin Microbiol Rev* 16: 242–264
- 43 Treanor J, Falsey A (1999) Respiratory viral infections in the elderly. *Antivir Res* 44: 79–102
- 44 Farha T, Thomson AH (2005) The burden of pneumonia in children in the developed world. *Paediatr Respir Rev* 6: 76–82
- 45 Lieberman D, Lieberman D, Gelfer Y, Varshavsky R, Dvoskin B, Leinonen M, Friedman MG (2002) Pneumonic vs nonpneumonic acute exacerbations of COPD. *Chest* 122: 1264–1270
- 46 Sato M, Saito R, Sakai T, Sano Y, Nishikawa M, Sasaki A, Shobugawa Y, Gejyo F, Suzuki H (2005) Molecular epidemiology of respiratory syncytial virus infections among children with acute respiratory symptoms in a community over three seasons. *J Clin Microbiol* 43: 36–40
- 47 Elliott MB, Pryharski KS, Yu Q, Boutilier LA, Campeol N, Melville K, Laughlin TS, Gupta CK, Lerch RA, Randolph VB, LaPierre NA, Dack KM, Hancock GE (2004) Characterization of recombinant respiratory syncytial viruses with the region responsible for type 2 T-cell responses and pulmonary eosinophilia deleted from the attachment (G) protein. *J Virol* 78: 8446–8454
- 48 Peret TC, Hall CB, Schnabel KC, Golub JA, Anderson LJ (1998) Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. *J Gen Virol* 79 ( Pt 9): 2221–2229
- 49 Hacimustafaoglu M, Celebi S, Aynaci E, Sinirtas M, Koksall N, Kucukerdogan A, Ercan I, Goral G, Ildirim I (2004) The progression of maternal RSV antibodies in the offspring. *Arch Dis Child* 89: 52–53
- 50 Kaneko M, Watanabe J, Ueno E, Hida M, Sone T (2001) Risk factors for severe respiratory syncytial virus-associated lower respiratory tract infection in children. *Pediatr Int* 43: 489–492
- 51 Virkki R, Juven T, Rikalainen H, Svedstrom E, Mertsola J, Ruuskanen O

- (2002) Differentiation of bacterial and viral pneumonia in children. *Thorax* 57: 438–441
- 52 Openshaw PJ, Tregoning JS (2005) Immune responses and disease enhancement during respiratory syncytial virus infection. *Clin Microbiol Rev* 18: 541–555
- 53 Schmidt AC, Couch RB, Galasso GJ, Hayden FG, Mills J, Murphy BR, Chanock RM (2001) Current research on respiratory viral infections: third international symposium. *Antivir Res* 50: 157–196
- 54 Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE (2005) Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 352: 1749–1759
- 55 Falsey AR, Walsh EE (2005) Respiratory syncytial virus infection in elderly adults. *Drugs Aging* 22: 577–587
- 56 Falsey AR, Walsh EE (2000) Respiratory syncytial virus infection in adults. *Clin Microbiol Rev* 13: 371–384
- 57 Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE (1969) An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* 89: 405–421
- 58 Hall CB, McBride JT, Gala CL, Hildreth SW, Schnabel KC (1985) Ribavirin treatment of respiratory syncytial viral infection in infants with underlying cardiopulmonary disease. *JAMA* 254: 3047–3051
- 59 van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de GR, Fouchier RA, Osterhaus AD (2001) A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 7: 719–724
- 60 Ulloa-Gutierrez R, Skippen P, Synnes A, Seear M, Bastien N, Li Y, Forbes JC (2004) Life-threatening human metapneumovirus pneumonia requiring extracorporeal membrane oxygenation in a preterm infant. *Pediatrics* 114: e517–e519
- 61 Maggi F, Pifferi M, Vatteroni M, Fornai C, Tempestini E, Anzilotti S, Lanini L, Andreoli E, Ragazzo V, Pistello M, Specter S, Bendinelli M (2003) Human metapneumovirus-associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. *J Clin Microbiol* 41: 2987–2991
- 62 Boivin G, De SG, Cote S, Gilca R, Abed Y, Rochette L, Bergeron MG, Dery P (2003) Human metapneumovirus infections in hospitalized children. *Emerg Infect Dis* 9: 634–640
- 63 Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, Wright PF, Crowe JE, Jr (2004) Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 350: 443–450
- 64 Hamelin ME, Cote S, Laforge J, Lampron N, Bourbeau J, Weiss K, Gilca R, DeSerres G, Boivin G (2005) Human metapneumovirus infection in adults with community-acquired pneumonia and exacerbation of chronic obstructive pulmonary disease. *Clin Infect Dis* 41: 498–502
- 65 Wyde PR, Chetty SN, Jewell AM, Boivin G, Piedra PA (2003) Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin *in vitro*. *Antiviral Res* 60: 51–59

- 66 Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den HB, Osterhaus AD, Ruuskanen O (2004) Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 10: 1095–1101
- 67 Legg JP, Warner JA, Johnston SL, Warner JO (2005) Frequency of detection of picornaviruses and seven other respiratory pathogens in infants. *Pediatr Infect Dis J* 24: 611–616
- 68 Papadopoulos NG (2004) Do rhinoviruses cause pneumonia in children? *Paediatr Respir Rev* 5 (Suppl A): S191-S195
- 69 Kellner G, Popow-Kraupp T, Kundi M, Binder C, Wallner H, Kunz C (1988) Contribution of rhinoviruses to respiratory viral infections in childhood: a prospective study in a mainly hospitalized infant population. *J Med Virol* 25: 455–469
- 70 Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA (2000) Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. *Eur Respir J* 16: 677–683
- 71 Rotbart HA, Hayden FG (2000) Picornavirus infections: a primer for the practitioner. *Arch Fam Med* 9: 913–920
- 72 Boyd MT, Jordan SW, Davis LE (1987) Fatal pneumonitis from congenital echovirus type 6 infection. *Pediatr Infect Dis J* 6: 1138–1139
- 73 Toce SS, Keenan WJ (1988) Congenital echovirus 11 pneumonia in association with pulmonary hypertension. *Pediatr Infect Dis J* 7: 360–362
- 74 Cheeseman SH, Hirsch MS, Keller EW, Keim DE (1977) Fatal neonatal pneumonia caused by Echovirus type 9. *Am J Dis Child* 131: 1169
- 75 Ventura KC, Hawkins H, Smith MB, Walker DH (2001) Fatal neonatal echovirus 6 infection: autopsy case report and review of the literature. *Mod Pathol* 14: 85–90
- 76 Chang LY, Lin TY, Hsu KH, Huang YC, Lin KL, Hsueh C, Shih SR, Ning HC, Hwang MS, Wang HS, Lee CY (1999) Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. *Lancet* 354: 1682–1686
- 77 Ledford RM, Collett MS, Pevear DC (2005) Insights into the genetic basis for natural phenotypic resistance of human rhinoviruses to pleconaril. *Antiviral Res* 68: 135–138
- 78 Rotbart HA (2002) Treatment of picornavirus infections. *Antiviral Res* 53: 83–98
- 79 Lednicky JA (2003) Hantaviruses. a short review. *Arch Pathol Lab Med* 127: 30–35
- 80 Zeier M, Handermann M, Bahr U, Rensch B, Muller S, Kehm R, Muranyi W, Darai G (2005) New ecological aspects of hantavirus infection: a change of a paradigm and a challenge of prevention – a review. *Virus Genes* 30: 157–180
- 81 Miedzinski L (2005) Community-acquired pneumonia: new facets of an old disease – Hantavirus pulmonary syndrome. *Respir Care Clin N Am* 11: 45–58
- 82 Ferres M, Vial P (2004) Hantavirus infection in children. *Curr Opin Pediatr* 16: 70–75
- 83 Gremillion DH, Crawford GE (1981) Measles pneumonia in young adults. An analysis of 106 cases. *Am J Med* 71: 539–542

- 84 Kim EA, Lee KS, Primack SL, Yoon HK, Byun HS, Kim TS, Suh GY, Kwon OJ, Han J (2002) Viral pneumonias in adults: radiologic and pathologic findings. *Radiographics* 22 (Spec No): S137–S149
- 85 Chiba ME, Saito M, Suzuki N, Honda Y, Yaegashi N (2003) Measles infection in pregnancy. *J Infect* 47: 40–44
- 86 Drut R, Drut RM (1988) Measles pneumonia in a newborn. *Pediatr Pathol* 8: 553–557
- 87 Frederick DM, Bland D, Gollin Y (2002) Fatal disseminated herpes simplex virus infection in a previously healthy pregnant woman. A case report. *J Reprod Med* 47: 591–596
- 88 Ramsey PG, Fife KH, Hackman RC, Meyers JD, Corey L (1982) Herpes simplex virus pneumonia: clinical, virologic, and pathologic features in 20 patients. *Ann Intern Med* 97: 813–820
- 89 Jones AM, Thomas N, Wilkins EG (2001) Outcome of varicella pneumonitis in immunocompetent adults requiring treatment in a high dependency unit. *J Infect* 43: 135–139
- 90 Gasparetto EL, Warszawiak D, Tazoniero P, Escuissato DL, Marchiori E (2005) Varicella pneumonia in immunocompetent adults: report of two cases, with emphasis on high-resolution computed tomography findings. *Braz J Infect Dis* 9: 262–265
- 91 Chou DW, Lee CH, Chen CW, Chang HY, Hsiue TR (1999) Varicella pneumonia complicated by acute respiratory distress syndrome in an adult. *J Formos Med Assoc* 98: 778–782
- 92 Goodnight WH, Soper DE (2005) Pneumonia in pregnancy. *Crit Care Med* 33: S390–S397
- 93 Harger JH, Ernest JM, Thurnau GR, Moawad A, Momirova V, Landon MB, Paul R, Miodovnik M, Dombrowski M, Sibai B, Van DP (2002) Risk factors and outcome of varicella-zoster virus pneumonia in pregnant women. *J Infect Dis* 185: 422–427
- 94 Pfeiffer H, Varchmin-Schultheiss K, Brinkmann B (2006) Sudden death in childhood due to varicella pneumonia: a forensic case report with clinical implications. *Int J Legal Med* 120: 33–35
- 95 Brayer C, Bony C, Salles M, Samperiz S, Pilorget H, Attali T, Alessandri JL (2004) [Bronchopulmonary dysplasia and cytomegalovirus pneumonia]. *Arch Pediatr* 11: 223–225
- 96 Sawyer MH, Edwards DK, Spector SA (1987) Cytomegalovirus infection and bronchopulmonary dysplasia in premature infants. *Am J Dis Child* 141: 303–305
- 97 Suzumura H, Sakurai K, Kano K, Ichimura T (1996) Chronic respiratory failure after acquired cytomegalovirus infection in a very low birthweight infant. *Acta Paediatr Jpn* 38: 677–680
- 98 Pedrazzini AG, Petralli C, Pedrazzini A, Luscieti P, Pedrinis E, Kauzlaric D (1981) [Cytomegalovirus pneumonia in primarily healthy adults]. *Schweiz Med Wochenschr* 111: 943–947
- 99 Chemaly RF, Torres HA, Hachem RY, Noguera GM, Aguilera EA, Younes A, Luna MA, Rodriguez G, Tarrand JJ, Raad II (2005) Cytomegalovirus pneumonia in patients with lymphoma. *Cancer* 104: 1213–1220

- 100 Knox KK, Pietryga D, Harrington DJ, Franciosi R, Carrigan DR (1995) Progressive immunodeficiency and fatal pneumonitis associated with human herpesvirus 6 infection in an infant. *Clin Infect Dis* 20: 406–413
- 101 Cone RW, Huang ML, Hackman RC (1994) Human herpesvirus 6 and pneumonia. *Leuk Lymphoma* 15: 235–241
- 102 Plummer G, Benyesh-Melnick M (1964) A plaque reduction neutralization test for human cytomegalovirus. *Proc Soc Exp Biol Med* 117: 145–150
- 103 Merk J, Schmid FX, Fleck M, Schwarz S, Lehane C, Boehm S, Salzberger B, Birnbaum DE (2005) Fatal pulmonary failure attributable to viral pneumonia with human herpes virus 6 (HHV6) in a young immunocompetent woman. *J Intensive Care Med* 20: 302–306
- 104 Gautschi O, Berger C, Gubler J, Laube I (2003) Acute respiratory failure and cerebral hemorrhage due to primary Epstein-Barr virus infection. *Respiration* 70: 419–422
- 105 Ankermann T, Claviez A, Wagner HJ, Krams M, Riedel F (2003) Chronic interstitial lung disease with lung fibrosis in a girl: uncommon sequelae of Epstein-Barr virus infection. *Pediatr Pulmonol* 35: 234–238
- 106 Mueller GA, Pickoff AS (2003) Pediatric lymphocytic interstitial pneumonitis in an HIV-negative child with pulmonary Epstein-Barr virus infection. *Pediatr Pulmonol* 36: 447–449
- 107 Kaan PM, Hegele RG, Hayashi S, Hogg JC (1997) Expression of bcl-2 and Epstein-Barr virus LMP1 in lymphocytic interstitial pneumonia. *Thorax* 52: 12–16
- 108 Marzouk K, Corate L, Saleh S, Sharma OP (2005) Epstein-Barr-virus-induced interstitial lung disease. *Curr Opin Pulm Med* 11: 456–460
- 109 Oda Y, Okada Y, Katsuda S, Nakanishi I (1994) Immunohistochemical study on the infection of herpes simplex virus, human cytomegalovirus, and Epstein-Barr virus in secondary diffuse interstitial pneumonia. *Hum Pathol* 25: 1057–1062
- 110 Pflieger A, Eber E, Popper H, Zach MS (2000) Chronic interstitial lung disease due to Epstein-Barr virus infection in two infants. *Eur Respir J* 15: 803–806
- 111 Balfour-Lynn IM, Girdhar DR, Aitken C (1995) Diagnosing respiratory syncytial virus by nasal lavage. *Arch Dis Child* 72: 58–59
- 112 Heikkinen T, Marttila J, Salmi AA, Ruuskanen O (2002) Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol* 40: 4337–4339
- 113 Falsey AR, Formica MA, Walsh EE (2002) Diagnosis of respiratory syncytial virus infection: comparison of reverse transcription-PCR to viral culture and serology in adults with respiratory illness. *J Clin Microbiol* 40: 817–820
- 114 Akhtar N, Ni J, Stromberg D, Rosenthal GL, Bowles NE, Towbin JA (1999) Tracheal aspirate as a substrate for polymerase chain reaction detection of viral genome in childhood pneumonia and myocarditis. *Circulation* 99: 2011–2018
- 115 Simpson JL, Moric I, Wark PA, Johnston SL, Gibson PG (2003) Use of induced sputum for the diagnosis of influenza and infections in asthma: a comparison of diagnostic techniques. *J Clin Virol* 26: 339–346
- 116 Xiang X, Qiu D, Chan KP, Chan SH, Hegele RG, Tan WC (2002) Comparison of three methods for respiratory virus detection between induced sputum

- and nasopharyngeal aspirate specimens in acute asthma. *J Virol Methods* 101: 127–133
- 117 Jain P, Sandur S, Meli Y, Arroliga AC, Stoller JK, Mehta AC (2004) Role of flexible bronchoscopy in immunocompromised patients with lung infiltrates. *Chest* 125: 712–722
- 118 Woo PC, Lau SK, Wong BH, Tsoi HW, Fung AM, Kao RY, Chan KH, Peiris JS, Yuen KY (2005) Differential sensitivities of severe acute respiratory syndrome (SARS) coronavirus spike polypeptide enzyme linked immunosorbent assay (ELISA) and SARS coronavirus nucleocapsid protein ELISA for serodiagnosis of SARS coronavirus pneumonia. *J Clin Microbiol* 43: 3054–3058
- 119 Niedrig M, Leitmeyer K, Lim W, Peiris M, Mackenzie JS, Zambon M (2005) First external quality assurance of antibody diagnostic for SARS-new coronavirus. *J Clin Virol* 34: 22–25
- 120 Gregson D, Lloyd T, Buchan S, Church D (2005) Comparison of the RSV respi-strip with direct fluorescent-antigen detection for diagnosis of respiratory syncytial virus infection in pediatric patients. *J Clin Microbiol* 43: 5782–5783
- 121 Brandenburg AH, Groen J, van Steensel-Moll HA, Claas EC, Rothbarth PH, Neijens HJ, Osterhaus AD (1997) Respiratory syncytial virus specific serum antibodies in infants under six months of age: limited serological response upon infection. *J Med Virol* 52: 97–104
- 122 Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H (2005) Detection of human metapneumovirus antigens in nasopharyngeal secretions by an immunofluorescent-antibody test. *J Clin Microbiol* 43: 1138–1141