Abstract

Acute respiratory viral infections (ARVI’s) are the most common infectious disease in humans. With the appearance of molecular techniques the recovery of viruses has dramatically increased. Nowadays virologists can quickly discriminate virological families and related viruses from emerging viruses and consequently identify novel viruses. Many new respiratory viruses have been identified in children in the past 15 years. In this review we shortly discuss novel respiratory viruses and their pathogenic role in pediatric respiratory disease. Advantages and drawbacks of the technique and our current knowledge will be discussed. We will conclude this review with a general discussion on the future role of molecular diagnostic virology in the clinic.

16.1 Introduction

16.1.1 General (Respiratory Viruses in Pediatrics)

Acute respiratory viral infections (ARVI’s) are the most common infectious disease in humans. They occur more frequently in children than in adults (6.1 episodes per year under the age of 1, 3–6 episodes per year between the age of 1–5 and 2.4 episodes per year between the age of 15–19). Disease severity depends on age, underlying condition and type of virus. ARVI’s account for huge numbers of doctor’s visits and days lost from work and school. They are a leading cause of global mortality and morbidity in children. Moreover, respiratory viral infections are an important driver of unnecessary usage of antibiotics. Unfortunately prevention and treatment of the majority of respiratory virus infections is not possible with the exception of influenza [1].

Although much research has been done on the epidemiology and burden of viral respiratory tract infections the size of the problem is underestimated. Due to the lack of routine testing for (multiple) viruses and the limitation that a majority of infected patients will not visit a doctor.
With the appearance of molecular techniques the recovery of viruses has dramatically increased. Before their use approximately 50–80% of the viral tests remained negative [2]. Due to the introduction of PCR and the discovery of novel viruses this proportion decreased to 3–15%. However, such recovery rates are largely dependent on the selection of the patient group [3–5]. The combination of high sensitivity, multiplex options and quantification was essential for some of the new insights in viral epidemiology. This could not have been achieved with conventional viral diagnostics such as culture and immunofluorescence assays (FDA).

The identification of respiratory viruses in a clinical context can also guide diagnostic and treatment strategies. Bonner et al. [6] revealed that a known viral aetiology of disease results in decreased use of additional tests such as X-rays or blood examination, shorter hospital admission and less frequent use of antibiotics. However, molecular diagnostics have also created new dilemmas. For example, the identification of respiratory viruses in asymptomatic children, the occurrence of many viral co-infections, concerns about the pathogenic capacity of certain viruses and the value of quantitative measurements.

Some of the advantages of the new genetic (e.g. sequencing) and molecular techniques became clear during outbreaks of novel emerging viruses. Emerging viruses can be classified as (1) previously unknown viruses or (2) previously known viruses that have significantly increased in incidence [7]. Nowadays virologists can quickly discriminate virological families and related viruses from emerging viruses and consequently identify novel viruses.

The introduction of molecular diagnostics in medical virology has led to the identification of many new respiratory viruses in children in the past 15 years (Table 16.1). However, the pathogenicity of these viruses is not always clear and the clinical relevance is often poorly understood. Fredricks and Relman proposed seven rules which are necessary to demonstrate the causative relationship between a virus and disease. These rules are based on Koch’s postulates and were adapted for nucleic acid based detection methods, location of the pathogen and quantification (Table 16.2) [8, 9]. These rules can help to interpret research on the role of novel respiratory viruses in disease and guide future research. It should also be stated that the clinical relevance is in some cases apparent, without extensive research to fulfil all requirements.

In this review we briefly discuss novel respiratory viruses and their pathogenic role in pediatric respiratory disease. We will conclude this review with general discussion on the future role of molecular diagnostic virology in the clinic.

### Table 16.1 Emerging viruses from the last 2 decades

| Virus Family | Year of discovery |
|--------------|-------------------|
| Hendra-/NipahV | 1995 |
| AIV’s | 1997 |
| hMPV | 2001 |
| SARS-CoV | 2003 |
| HCoV-NL63 | 2004 |
| HCoV-HKU1 | 2005 |
| HBoV | 2005 |
| HPeV4 | 2006 |
| HPeV5 | 2006 |
| HPeV6 | 2007 |
| KIV/WU | 2007 |
| H1N1V | 2009 |

### 16.2 Henipavirus (1994–1998)

#### 16.2.1 Hendra Virus

The Hendra virus was first detected in a disease outbreak in 1994. It initially presented with a new respiratory disease in horses that was transmitted to two persons one of them died [10]. The virus belongs to the genus of *Henipavirus* within...
the family of the Paramyxoviridae family. It was initially named morbillivirus and later re-named Hendra virus (HeV) after the suburb where the outbreak occurred [11, 12]. The virus itself is not very contagious. It spreads through direct contact between horses or during intensive contact between humans and severely ill horses. The animal reservoir appears to be the Flying-fox, in this population the Hendra infection is largely subclinical. The breeding season of Flying-foxes is a risk period for spread and the human risk group is defined as people with close and intensive contact with horses. Up till now fourteen outbreaks have been reported [13]. In five of these outbreaks humans were involved, resulting in five deaths. So far, only two persons survived an infection. The case fatality rate (CFR) is over 50% [13]. HeV in humans causes, after an incubation period of 5–21 days, a severe influenza like disease (fever, myalgia and headache) which can progress to pneumonia, respiratory failure and death [10, 14]. An infection can also result in encephalitis with headache, fever and drowsiness. The encephalitis can occur after initial recovery from the illness. The Hendra virus genome is readily detected in several materials, e.g. blood, urine, nasal- and oropharyngeal swabs by RT-PCR. Next to this standard detection method the virus can be cultured in several cell lines, where it forms syncytia upon infection. ELISA serological tests are used for screening, however their diagnostic sensitivity is not yet established. Immunofluorescence assays and serum neutralization methods can also be used [13, 15].

16.2.2 Nipah Virus

In 1998 and 1999 two large outbreaks of respiratory disease in pigs and humans occurred. In Malaysia and Singapore 106 people died [16]. The causative agent had large similarities to the HeV and is the second member of the genus of Henipavirus within the Paramyxoviridae family. It was named Nipah virus (NiV) after the location of the first human case [17]. This virus had already caused respiratory disease in pigs until late 1996. Like HeV it shares the bat as a natural reservoir. NiV virus is very contagious among pigs and spreads through the respiratory route or directly by the transport of infected pigs. Initially humans became infected via direct contact with pigs, although food borne transmissions were also reported. Initially the case fatality rate was 38.5% [14, 18]. Since the initial outbreak almost yearly new outbreaks emerged in Bangladesh and India causing fatal encephalitis in humans. Notably, the CFR’s of these outbreaks increased to 92%. Also the transmission changed: starting from pigs, soon cows could transmit the virus. Later human to human and nosocomial transmission was demonstrated [19–21]. It has been hypothesized that there were multiple introductions of viruses in the human population, explaining the unique genetic signature of isolated viruses nowadays [18]. These genetic differences may be the reason for the increase in CFR and differences in clinical manifestations.
and transmission. The clinical manifestation of a Nipah infection differed per outbreak. Incubation periods differ from an average of 2 weeks in Malaysia to 1 week in Bangladesh. The infection can be asymptomatic, but often starts with influenza-like symptoms of fever, headache, myalgia, vomiting and sore throat. Patients can recover or develop signs of encephalitis or sometimes atypical pneumonia or acute respiratory distress. In severe cases the encephalitis includes the brain stem or progresses to a coma within 24–48 h [18, 19, 22]. Around 20% of the cases are left with residual neurological symptoms, including personality changes. In comparison with outbreaks in Malaysia and Singapore the Bangladesh and Indian patients experienced more profound respiratory symptoms with case rates of 14, 27, 70 and 51%, respectively [19]. A Nipah infection can be diagnosed in serum urine and cerebrospinal fluid (CSF) by RT-PCR [23]. Also culture in cell-lines, ELISA for anti-HeV IgG and IgM in serum and CSF, serum neutralization assays or immunofluorescence assays are used [18, 19, 24, 25].

Patients with both Hendra and Nipah virus infections are treated supportive, antiviral therapy is not effective [22]. Prevention is based on careful hygiene, quarantine and safe disposal of animal carcasses [13, 14]. Currently, there are no vaccines available. However, several therapeutic agents seem effective in vitro and in some animal models [18].

16.2.3 Avian Influenza Virus (1997)

The first cases of avian influenza virus (AIV) infection were reported in 1997 in Honk-Kong [26]. This influenza A (H5H1) originated completely from strains circulating in wild birds and poultry [27]. The avian influenza virus undergoes rapid genetic and antigenic evolution reflected by the occurrence of different clades with distinct phenotypes [28, 29]. The majority of human cases had direct contact with poultry or could be related to outbreaks in wild birds [20]. There is limited transmission from human to human, although some epidemiological studies suggest it is possible [30]. The median age of patients is around 18 years and the mortality rate is extremely high between the age of 10 and 19 years (61%). Yearly H5N1 outbreaks in humans have been reported in Asia, Africa and Eurasia [31]. These epidemics are all related to outbreaks of avian influenza in wild birds or poultry during the colder seasons [32, 33]. The incubation period of H5N1 is estimated to be 2–7 days [34]. The disease typically manifests as a severe pneumonia which often progresses to respiratory failure and death within 10 days (case fatality rate up to 90% in children). It appears that in children cases may occur without pneumonia. Detection of viral RNA by (RT-) PCR is the best method for the diagnosis of H5N1, preferably using throat swabs [35]. Because of genetic variability of the virus, primers need to be updated frequently. The available immune-assays for detection of H5N1 are not sensitive enough for clinical purposes and cannot differentiate between human and avian subtypes of influenza A. Seroconversion after 2–3 weeks can be used to confirm H5N1 infection and can be used for epidemiological studies [35]. Early treatment with oseltamivir is recommended based on some evidence that it increases survival rates [36]. There are differences in susceptibility to oseltamivir between the different clades of H5N1 circulating in different parts of the world. Combination of oseltamivir with amantidine can be given if the circulating H5N1 is susceptible to both agents. Currently, it is possible to produce vaccines that inactivate H5 influenza A strains. However due to the circulation of different clades and the rapidly changing antigenicity of H5N1 the need for the development of a new vaccine remains [34, 37].

16.2.4 Metapneumovirus (2001)

The human metapneumovirus (hMPV) was first discovered in the Netherlands in 2001 from a databank of samples from children with respiratory tract infections [38]. hMPV belongs to the genus *Metapneumovirus* within the family of Paramyxoviridae. It is related to respiratory
syncytial virus, both belonging to the *pneumoviridae* sub-family. In both retrospective and prospective studies it has been shown that hMPV can be detected in 3.9–14.8% of respiratory samples from children with respiratory disease [39]. This wide range reflects differences in the tested populations and the level of care. Co-infections with other viruses occur in 15–30% [40–42]. hMPV is detected in up to 4% of nasopharyngeal aspirates from healthy children, although percentages of less than 1% are also frequently published [43]. Serological studies showed that all children by the age of 5 years had been in contact with the virus and that it has been circulating in the human population for over 50 years [38]. It has a seasonal occurrence with a peak incidence just after the influenza and RSV season [43]. Spread is thought to be via direct or close contact with respiratory secretions from an infected person with an incubation period of 3–5 days. Re-infections occur frequently in children, although symptoms are less severe [44]. Symptoms associated with hMPV infections are comparable with RSV (see Table 16.3). hMPV infections are, after RSV, the most frequent cause of bronchiolitis in young children and account for 5–15% of all hospital admissions [45]. Hospitalization rates are highest among 6–12 month old children, remarkably older than for RSV [46]. There is an association between severe hMPV infection (bronchiolitis) and the development of wheezing in childhood [47].

hMPV can only be cultured in specific cell lines under specific conditions and is time consuming; therefore it has no role in a clinical setting. Real time PCR is the most sensitive test for hMPV detection in NPA and swabs [48] and is therefore the common method in clinical and research settings. RT-PCR also provides semi-quantitative information of the viral load (Ct value), which can be used to monitor treatment in a research setting [49]. Immunofluorescence assays are available for rapid detection of the virus in respiratory specimens; however, these tests are less sensitive than RT-PCR. Serology for hMPV has little additive value in the clinic because most children are seropositive in early childhood. Currently no vaccines against hMPV are available, though several candidates are being pursued [45]. Ribavirin, antiviral therapy, is effective in vitro against hMPV, though clinical data are sparse. Currently new therapies such as fusion inhibitors and siRNA’s are being tested in murine models [45].

### 16.2.5 Coronavirus (2003–2005)

Human corona viruses related to respiratory disease, 229E and OC43, have been known since the 1930s. They were recognized as the second most common cause of the common cold in humans [50–52]. A new strain of human corona virus was identified in 2004 from a respiratory sample of a 7 month old infant with bronchiolitis and named NL63 (HCoV-NL63) [53]. The HCoV-NL63 belongs to the genus Coronavirus within the family of Coronavidae. In retrospective cohort studies HCoV-NL63 have been identified in 1.7–9.3% of respiratory samples from children with respiratory symptoms and occurs worldwide [39]. The virus is often found in combination with other respiratory viruses (57%) [54, 55]. Peak incidence is found in the winter months and the incubation period is estimated

| Symptoms/diagnosis | Spread in literature | a Heikkinen et al. [121]; Aberle et al. [122]; Mullins et al. [123]; Chen et al. [124]; Manoha et al. [125]; Williams et al. [126]; Bosis et al. [127] | b Different definitions varying from >37.5 to >39 °C |
|--------------------|----------------------|-------------------------------------------------|-------------------------------------------------|
| Feverb             | 36–80                |                                                 |                                                 |
| Cough              | 67–99                |                                                 |                                                 |
| Rhinitis           | 72–90                |                                                 |                                                 |
| Wheezing           | 10–73                |                                                 |                                                 |
| Respiratory failure| 8                    |                                                 |                                                 |
| Oxygen 90 %        | 32–85                |                                                 |                                                 |
| Pharyngitis        | 24–66                |                                                 |                                                 |
| Bronchitis         | 1–68                 |                                                 |                                                 |
| Bronchiolitis      | 11–51                |                                                 |                                                 |
| Pneumonia          | 3–65                 |                                                 |                                                 |
| Otitis media       | 16                   |                                                 |                                                 |

*Table 16.3 Symptoms and diagnosis of hMPV mono infections in literature*
2–5 days [56, 57]. HCoV-NL63 is associated with mild upper respiratory tract symptoms and less frequent with severe lower respiratory tract symptoms such as bronchiolitis [58]. Some studies have reported an association with croup [55, 59]. HCoV-NL can be detected in respiratory specimens by RT-PCR which is the first choice for diagnosis. Immunoassays are available for rapid detection and distinction of different HCoV strains [60]. Different cell-lines are permissive for viral culture and used in a research setting. Currently no anti-viral treatment against HCoV-NL63 is available, although several inhibiting compounds have been identified [61].

A second novel human coronavirus was identified in 2005 in a 71-year-old man with pneumonia in China and named HKU1 after the Hong Kong University where it was found [62]. In a retrospective cohort studies the HCoV-HKU1 was identified in 1–3.1 % of respiratory samples in which no other virus was detected, from children with upper and lower respiratory symptoms [39] with a higher incidence in children younger than 6 months. The peak incidence of HCoV-HKU1 is in spring, early summer and winter with an incubation period of 2 days [63]. HCoV-HKU1 is mainly associated with upper respiratory tract symptoms in children and occasionally with pneumonia and bronchiolitis [64]. The first choice of assay for detection in respiratory specimens is RT-PCR. Coronaviruses exhibit substantial genetic variability hampering the development of panco RNA primers and therefore specific primers for each strain have to be used [65]. There is no specific anti-viral therapy available against HCoV-HKU1.

### 16.2.6 Human Bocavirus (2005)

Human bocavirus (HBoV) belongs to the genus *Bocavirus* within the family *Parvoviridae* (and is closely related to the bovine parvovirus and canine minute virus). This virus was identified in 2005 by nucleic acid amplification (PCR) in respiratory tract specimens from Swedish children with lower respiratory tract infections [66]. In this study HBoV was detected in 3.1 % of hospitalized children below the age of three. Other studies detected HBoV in 3–19 % of children with respiratory symptoms depending on the sample type used (NPA and BAL *higher*, nasal swab *lower*) [67] and the age of the patient (*higher* in younger children) [68]. However, HBoV is frequently found in asymptomatic children (up to 40 %) or in combination with other viruses (up to 80 %) in symptomatic children [39]. Based on these findings it is still unclear whether HBoV has a pathogenic role in respiratory disease. One study performed in a PICU suggests that the viral load (high titres) of HBoV may indicate a pathogenic role in (severe) respiratory disease [69]. HBoV has been associated with wheezing in asthmatic children [70]. In general HBoV infection is marked by relatively mild symptoms of the upper respiratory tract such as cough, rhinorrhea and fever. In rare cases it has been associated with lower respiratory tract infection and even respiratory insufficiency [71]. Detection of HBoV is by RT-PCR and the virus can be detected in respiratory as well as gastrointestinal specimens [72]. Diagnostic seroresponses can be used to establish the specific immune response against HBoV during infection, although the clinical relevance is unclear [73]. HBoV can only be cultured on ciliated primary human epithelial cell-lines, and therefore viral isolation is only used in experimental settings [74]. Treatment of HBoV infections is mainly supportive and no specific anti-viral treatment against HBoV is available. Currently there is not enough epidemiological evidence to drive vaccine development against HBoV.

### 16.2.7 Parechovirus (2006–2007)

Human parechoviruses (HPEVs) belong to the genus *Parechovirus* of the family *Picornaviridae*. The first HPEVs, serotype 1 and 2, were identified 50 years ago during a summer diarrhoea outbreak in American children [75]. With the introduction of molecular techniques many new serotypes of HPEVs have been identified in the past 15 years in the stool or NPA of children with gastrointestinal and respiratory disease, and in the cerebrospinal
Fluid of children with meningitis and sepsis-like illness (see Table 16.4) [76–79]. Every HPeV serotype has its specific epidemiological and clinical features. All HPEVs infections are very common in children under the age of 1 year and most data are available on HPeV1 and HPeV3 [80]. The median age of infection with HPeV1 is 6.6 months, whereas HPeV3 infections occur at a younger age of 1.3 months. There is also seasonal variability in occurrence, HPeV1 in late summer and early winter season, and HBeV3 mostly in summer. HBeV serotype 5 and 6 have also been associated with respiratory tract symptoms [81–84].

Most HPeVs have are common causes of asymptomatic infection in early childhood and are often found in combination with other viruses, so that the relation with respiratory disease is hard to establish [80]. While the association of HPeV3 with encephalitis, meningitis and neonatal sepsis is widely accepted [85], for most other serotypes the relationship with disease and specific symptoms is less clear (see Table 16.4) [86].

A viral neutralisation assay or culture are time-consuming and not suitable for severe disease such as sepsis and meningitis. Detection by RT-PCR is only available for HPeV1-3 [87]. Currently amplification and nucleotide sequencing is used to identify specific genotypes in a research setting. The specific antibody response can be used to demonstrate involvement of HPeV in disease if the virus itself cannot be detected. No antiviral treatment against parechoviruses is currently available and only supportive care is given.

### 16.2.8 Polyomavirus (2007)

In 2007 two new members of the Polyomaviridae family were discovered in samples of patients with respiratory disease. The first of these new polyomaviruses was identified during a large scale molecular virus screening project in respiratory samples from children and named after the Karolinska institute where it was discovered (KIV) [88]. The second was identified in a nasopharyngeal aspirate of a 3-year-old child with pneumonia and named Washington University virus (WUV) [89]. Seroprevalence studies show KI in 66% and WU in 79% of paediatric sera [46, 90]. The virus has been detected in 1–5% respiratory samples worldwide in respiratory samples of young symptomatic children [91–93]. However, in 70–80% of the cases there was a co-infection with other respiratory viruses, and KIV and WUV have been described in asymptomatic HSCT patients [94]. Based on these results it is difficult to assign symptoms and pathogenicity to both of them and more epidemiological evidence is needed. In most studies the viruses have been associated with both upper and lower respiratory tract infections in children. Detection of WUV and KIV in respiratory samples can be undertaken by RT-PCR. Thus far there is no indication for treatment of either of these viruses nor vaccine development.

### 16.2.9 Influenza A H1N1 Virus (2009)

In late march 2009 a novel influenza A (H1N1) virus was identified in America. This virus was subsequently recognised as the cause of an outbreak of respiratory illness in Mexico [95]. The
novel flu virus showed reassortment of swine, avian and human strains, and appeared to be very infectious between humans [96]. After the initial detection several other countries reported H1N1 infections. In June 2009 the WHO declared a pandemic with spread over at least two continents. At the start of the pandemic the virus appeared to be very virulent with a high mortality rate, especially in young adults and children [97, 98]. However, in the Northern Hemisphere the virus behaved more like a seasonal influenza virus. H1N1 disease had the highest attack rate in young children causing relatively mild disease [99]. The pH1N1 was able to outcompete the seasonal flu so that, in the influenza season 2009–2010, over 99% of the influenza positive isolates in Europe and America were pandemic H1N1 influenza A [101].

In general the symptoms resembled those of other winter viruses: fever, cough, sore throat, myalgia and headache. Symptoms at presentation for hospitalised patients are shown in Table 16.5. Spread occurs up to 8 days after the start of symptoms although this may be prolonged in immunocompromised patients and children [102].

H1N1 infection can be diagnosed by RT-PCR on respiratory samples and this appears to be the most sensitive method. In case of high suspicion of H1N1 infection with a negative PCR result, the virus can be cultured or infection proven by documenting seroconversion [103–105].

During the pandemic of H1N1 were treated with oseltamivir (Tamiflu®) and zanamivir. This treatment reduced the duration of symptoms, the occurrence of otitis media and progression into severe disease, especially when administered early in the course of disease [106]. Also the prophylactic use of anti viral agents is effective in reducing the occurrence of H1N1 infections in exposed individuals. However, oseltamivir and multi drug resistant viruses are emerging [107]. In several countries children have been vaccinated [108, 109]. H1N1 vaccination induced an effective and long lasting humoral immune response [108, 109]. The vaccine seemed to reduce the risk of infection and decreased severity of disease in children, however because of the rapid spread of the H1N1 pandemic most people were vaccinated during the pandemic making efficacy studies complex [111, 112].

### Table 16.5 Symptoms of H1N1

| Presentation                        | Literaturea (%) |
|------------------------------------|-----------------|
| Fever (>38 °C)                     | 81–94           |
| Cough                              | 69–82           |
| Gastro-intestinal symptoms         | 8–32            |
| Rhinorrhea                         | 31–62           |
| Diarrhea                           | 8–23            |
| Wheezing                           | 12–25           |

a Libster et al. [97], Hackett et al. [128], Jain et al. [129]

In this review we have discussed newly identified and emerging viruses from the past 2 decades. These viruses could be subdivided in three categories, based on the evidence for their pathogenicity in respiratory disease in children. First, emerging viruses causing epidemics with high mortality, such as AIV, Hendra and Nipah virus, were clearly associated with a pathogenic role in disease. These epidemic-causing viruses are often of zoonotic origin (transmission from animals to humans). The second group comprises viruses that fulfil the modified Koch’s postulates [8, 9]. Most novel respiratory viruses are not completely characterised according to the postulates due to the extensive and costly research needed to achieve this. In this perspective hMPV is unique among the recently discovered respiratory viruses, because all criteria have been fulfilled [111]. Third are viruses that were found during screening for new respiratory viruses in respiratory samples with molecular techniques, such as human bocavirus, the novel polyomaviruses, parechoviruses and some coronaviruses. For most of these viruses their pathogenic role as an important respiratory pathogen is less clear. Although these viruses are present in respiratory
samples of children with respiratory disease however, they are also often present in asymptomatic children or found in combination with other viruses. Many studies were performed retrospectively, or without the proper control cohorts of asymptomatic children. In epidemiological studies based on seroconversion it is apparent that a first encounter with these viruses occurs early in childhood without (severe) respiratory tract infections. Especially in this last category of viruses, in which the association with respiratory disease is less clear, large prospective epidemiological studies are needed to further specify the pathogenicity and health burden of these viruses in children.

The highly sensitive molecular techniques for identification and detection of novel viruses are a powerful tool for epidemiological studies, especially when used in multiplex platforms. Their ability to quantify the viral burden in infection may be used as additional information in determining the role of a virus in respiratory disease. For some viruses a positive correlation between viral load and disease severity is described [69, 115]. However whether viral load correlates with disease severity in general remains a point of debate. Viral load appears to be lower in viral-viral co-infection compared to viral-mono-infections, the mechanism behind this and the clinical relevance requires further investigation [116, 117]. Studies show that viral load decreases during the course of disease, and this can be used as marker for the therapeutic effect of anti-viral compounds. A drawback of the high sensitivity of molecular diagnostics is that PCR signals remain positive after recovery from an illness, sometimes even for several weeks. Because young children have frequent viral infections of the upper respiratory tract, the value of a positive PCR test can be limited.

Interaction of viruses with bacteria present in the nasopharynx can result in enhanced disease severity. This is well known for influenza and Streptococcus pneumoniae, and other respiratory bacteria [118]. How other (novel) respiratory viruses interact with bacteria and how this leads to enhanced disease is less well known. In study-
monia of horses and its transmission to humans. Emerg Infect Dis 1:31–33
12. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L et al (1995) A morbillivirus that caused fatal disease in horses and humans. Science 268:94–97
13. Hess IMR, Massey PD, Walker B, Middleton DJ, Wright TM (2011) Hendra virus: what do we know? N S W Public Health Bull 22:118–122
14. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J (2001) The natural history of Hendra and Nipah viruses. Microbes Infect 3:307–314
15. Chiang CF, Lo MK, Rota PA, Spiropoulou CF, Rollin PE (2010) Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 7:115
16. CDC (1999) Outbreak of Hendra-like virus–Malaysia and Singapore, 1998–1999. MMWR Morb Mortal Wkly Rep 48:265–269
17. CDC (1999) Update: outbreak of Nipah virus–Malaysia and Singapore, 1999. MMWR Morb Mortal Wkly Rep 48:335–337
18. Lo MK, Rota PA (2008) The emergence of Nipah virus, a highly pathogenic paramyxovirus. J Clin Virol 43:396–400
19. WHO (2010) Nipah virus fact sheet (revised in July 2009). Wkly Epidemiol Rec 85:64–67
20. Mounts AW, Kwong H, Izurieta HS, Ho Y, Au T, Lee M et al (1999) Case-control study of risk factors for avian influenza A (H5N1) disease, Hong Kong, 1997. J Infect Dis 180:505–508
21. Tan CT, Tan KS (2001) Nosocomial transmissibility of Nipah virus. J Infect Dis 184:1367
22. Goh KJ, Tan CT, Chew NK, Tan PS, Kamarulzaman A, Sarji SA et al (2000) Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med 342:1229–1235
23. Guillaume V, Contamin H, Loth P, Georges-Courbot MC, Lefevre A, Marianneau P et al (2004) Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 78:834–840
24. Daniels P, Ksiazek T, Eaton BT (2001) Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 3:289–295
25. Bossart KN, McEachern JA, Hickey AC, Choudhry V, Dimitrov DS, Eaton BT et al (2007) Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. J Virol Methods 142:29–40
26. (1997) Isolation of avian influenza A(H5N1) viruses from humans–Hong Kong, May–December 1997. MMWR Morb Mortal Wkly Rep 46:1204–1207
27. Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H et al (1998) Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 279:393–396
28. Chen H, Smith GJ, Li KS, Wang J, Fan XH, Rayner JM et al (2006) Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc Natl Acad Sci U S A 103:2845–2850
29. Webster RG, Govorkova EA (2006) H5N1 influenza—continuing evolution and spread. N Engl J Med 355:2174–2177
30. Tran TH, Nguyen TL, Nguyen TD, Luong TS, Pham PM, Nguyen VC et al (2004) Avian influenza A (H5N1) in 10 patients in Vietnam. N Engl J Med 350:1179–1188
31. Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD et al (2005) Avian influenza A (H5N1) infection in humans. N Engl J Med 353:1374–1385
32. Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P (2006) Predicting the global spread of H5N1 avian influenza. Proc Natl Acad Sci U S A 103:19368–19373
33. Ducatze M, Olinger CM, Owodea AA, De Landsheer S, Ammerlaan W, Niesters HG et al (2006) Avian flu: multiple introductions of H5N1 in Nigeria. Nature 442:37
34. Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z, Hayden FG, Nguyen DH, de Jong MD et al (2008) Update on avian influenza A (H5N1) virus infection in humans. N Engl J Med 358:261–273
35. de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN et al (2006) Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med 12:1203–1207
36. Schunemann HJ, Hill SR, Kakad M, Bellamy R, Uyei TM, Hayden FG et al (2007) WHO Rapid Advice Guidelines for pharmacological management of sporadic human infection with avian influenza A (H5N1) virus. Lancet Infect Dis 7:21–31
37. Girard MP, Osterhaus A, Pervikov Y, Palkonyay L, Kieny MP (2008) Report of the third meeting on “influenza vaccines that induce broad spectrum and long-lasting immune responses”, World Health Organization, Geneva, Switzerland, 3–4 December 2007. Vaccine 26:2443–2450
38. van den Hoogen BG, van Doornum GJ, Fockens JC, Cornelissen JJ, Beyer WE, de Groot R et al (2003) Prevalence and Clinical Symptoms of Human Metapneumovirus Infection in Hospitalized Patients. The J Infect Dis 188:1571–1577
39. Brodzinski H, Ruddy RM (2009) Review of new and newly discovered respiratory tract viruses in children. Pediatr Emerg Care 25:352–360; quiz 61–3
40. Via佐 S, Ratjen F, Scheidhauer R, Fiedler M, Roggendorf M (2003) High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. J Clin Microbiol 41:3043–3045
41. Dollner H, Riske K, Radtke A, Nordbo SA (2004) Outbreak of human metapneumovirus infection in norwegian children. Pediatr Infect Dis J 23:436–440
Elucidation and Clinical Role of Emerging Viral Respiratory Tract Infections in Children

42. Maggi F, Pifferi M, Vatteroni M, Fornai C, Tempestini E, Anzilotti S et al (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

43. Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingerhaus MJ, Edwards KM et al (2004) Human Metapneumovirus and Lower Respiratory Tract Disease in Otherwise Healthy Infants and Children. N Engl J Med 350:443–450

44. Pelletier G, Dery P, Abed Y, Boivin G (2002) Respiratory tract reinfections by the new human Metapneumovirus in an immunocompromised child. Emerg Infect Dis 8:976–978

45. Kahn JS (2006) Epidemiology of Human Metapneumovirus. Clin Microbiol Rev 19:546–557

46. Papenburg J, Boivin G (2010) The distinguishing features of human metapneumovirus and respiratory syncytial virus. Rev Med Virol 20:245–260

47. Garcia DF, Hiatt PW, Jewell A, Schoonover SL, Cron SG, Rigg MS et al (2007) Human metapneumovirus and respiratory syncytial virus infections in older children with cystic fibrosis. Pediatr Pulmonol 42:66–74

48. Landry ML, Cohen S, Ferguson D (2008) Prospective study of human metapneumovirus detection in clinical samples by use of light diagnostics direct immunofluorescence reagent and real-time PCR. J Clin Microbiol 46:1098–1100

49. Maertzdorf J, Wang CK, Brown JB, Quinto JD, Chu M, de Graaf M et al (2004) Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. J Clin Microbiol 42:981–986

50. Tyrell DJ, Struve FA, Schwartz ML (1965) A methodological consideration in the performance of process and reactive schizophrenics on a test for organic brain pathology. J Clin Psychol 21:254–256

51. Hamre D, Procknow JI (1966) A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med 121:190–193

52. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM (1967) Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Natl Acad Sci U S A 57:933–940

53. van der Hoeck L, Pyrc K, Jebbink MF, Vermeulenk-Oost W, Berkhout RJ, Wolthers KC et al (2004) Identification of a new human coronavirus. Nat Med 10:368–373

54. Boivin G, Coulombe Z, Wat C (2003) Quantification of the influenza virus load by real-time polymerase chain reaction in nasopharyngeal swabs of patients treated with oseltamivir. J Infect Dis 188:578–580

55. van der Hoeck L, Sure K, Ihorst G, Stang A, Pyrc K, Jebbink MF et al (2005) Croup is associated with the novel coronavirus NL63. PLoS Med 2:e240

56. Bastien N, Robinson JL, Tse A, Lee BE, Hart L, Li Y (2005) Human coronavirus NL-63 infections in children: a 1-year study. J Clin Microbiol 43:4567–4573

57. Bastien N, Anderson K, Hart L, Van Caeseele P, Brandt K, Milley D et al (2005) Human coronavirus NL63 infection in Canada. J Infect Dis 191:503–506

58. Arden KE, Nissen MD, Sloots TP, Mackay IM (2005) New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. J Med Virol 75:455–462

59. Han TH, Chung JY, Kim SW, Hwang ES (2007) Human Coronavirus-NL63 infections in Korean children, 2004–2006. J Clin Virol 38:27–31

60. Gerna G, Vitulo P, Rovida F, Lilleri D, Pellegrini C, Oggioni T et al (2006) Impact of human metapneumovirus and human cytomegalovirus versus other respiratory viruses on the lower respiratory tract infections of lung transplant recipients. J Med Virol 78:408–416

61. Pfefferle S, Schopf J, Kogl M, Friedel CC, Muller MA, Carbauo-Lozoya J et al (2011) The SARS-Coronavirus-Host Interactome: Identification of Cyclophilins as Target for Pan-Coronavirus Inhibitors. PLoS Pathog 7:e1002331

62. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y et al (2005) Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 79:884–895

63. Ren L, Gonzalez R, Xu J, Xiao Y, Li Y, Zhou H et al (2011) Prevalence of human coronaviruses in adults with acute respiratory tract infections in Beijing, China. J Med Virol 83:291–297

64. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS (2006) Coronavirus HKU1 infection in the United States. Emerg Infect Dis 12:775–779

65. Vijgen L, Moeis E, Keyaerts E, Li S, Van Ranst M (2008) A pancoronavirus RT-PCR assay for detection of all known coronaviruses. Methods Mol Biol 454:3–12

66. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A 102:12891–12896

67. Longtin J, Bastien M, Gilca R, Leblanc E, de Serres G, Bergeron MG et al (2008) Human bocavirus infections in hospitalized children and adults. Emerg Infect Dis 14:217–221

68. Arnold JC, Singh KK, Spector SA, Sawyer MH (2006) Human bocavirus: prevalence and clinical spectrum at a children’s hospital. Clin Infect Dis 43:283–288

69. van de Pol AC, Wolfs TF, Jansen NJ, Kimpen JL, van Loon AM, Rossen JW (2009) Human bocavirus and KI/WU polyomaviruses in pediatric intensive care patients. Emerg Infect Dis 15:454–457
80. Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R et al (2007) Human bocavirus and acute wheezing in children. Clin Infec Dis 44:904–910
81. Allander T (2008) Human bocavirus. J Clin Virol 41:29–33
82. Lu X, Chittaganpitch M, Olsen SJ, Mackay IM, Sloots TP, Fry AM et al (2006) Real-time PCR assays for detection of bocavirus in human specimens. J Clin Microbiol 44:3231–3235
83. Soderlund-Venermo M, Lahtinen A, Jartti T, Hedman L, Kemppainen K, Lehtinen P et al (2009) Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis 15:1423–1430
84. Dijkman R, Koekkoek SM, Molenkamp R, Schil- den O, van der Hoek L (2009) Human bocavirus can be cultured in differentiated human airway epithelial cells. J Virol 83:7739–7748
85. Wigand R, Sabin AB (1961) Properties of ECHO types 22, 23 and 24 viruses. Arch Gesamte Virus- forsch 11:224–247
86. Baumgarte S, de Souza Luna LK, Grywna K, Panning M, Drexler JF, Karsten C et al (2008) Prevalence, types, and RNA concentrations of human parechoviruses, including a sixth parechovirus type, in stool samples from patients with acute enteritis. J Clin Microbiol 46:242–248
87. Benschop KS, Schinkel J, Luken ME, van den Broek PJ, Beersma MF, Menilik N et al (2006) Fourth human parechovirus serotype. Emerg Infect Dis 12:1572–1575
88. Al-Sunaidi M, Williams CH, Hughes PJ, Schnurr DP, Stanway G (2007) Analysis of a new human parechovirus allows the definition of parechovi- rus types and the identification of RNA structural domains. J Virol 81:1013–1021
89. Ito M, Yamashita T, Tszuki H, Takeda N, Saka- ke K (2004) Isolation and identification of a novel human parechovirus. J Gen Virol 85:391–398
90. Harvala H, Robertson I, McWilliam Leitch EC, Benschop K, Wolthers KC, Templeton K et al (2008) Epidemiology and clinical associations of human parechovirus respiratory infections. J Clin Microbiol 46:3446–3453
91. Schnurr D, Dondero M, Holland D, Connor J (1996) Characterization of echovirus 22 variants. Arch Virol 141:1749–1758
92. Watanabe K, Oie M, Higuchi M, Nishikawa M, Fujii M (2007) Isolation and characterization of novel human parechovirus from clinical samples. Emerg Infect Dis 13:889–895
93. Chieochansin T, Vichiwiattana P, Korkong S, Themboonlers A, Povorawan Y (2011) Molecular epidemiology, genome characterization, and recombination event of human parechovirus. Virology 421:159–166
94. Pajkrt D, Benschop KS, Westerhuis B, Molen- kamp R, Spanjerberg L, Wolthers KC (2009) Clinical characteristics of human parechoviruses 4–6 infections in young children. Pediatr Infect Dis J 28:1008–1010
95. Harvala H, Robertson I, Chieochansin T, McWill- iam Leitch EC, Templeton K, Simmonds P (2009) Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. J Infect Dis 199:1753–1760
96. Harvala H, Wolthers KC, Simmonds P (2010) Parechoviruses in children: understanding a new infection. Curr Opin Infect Dis 23:224–230
97. Nix WA, Maher K, Johansson ES, Niklasson B, Lindberg AM, Pallansch MA et al (2008) Detection of all known parechoviruses by real-time PCR. J Clin Microbiol 46:2519–2524
98. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA et al (2007) Identification of a third human polyomavirus. J Virol 81:4130–4136
99. Gaynor AM, Nissen MD, Whiteley DM, Mackay IM, Lambert SB, Wu G et al (2007) Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog 3:e64
100. Nguyen NL, Le BM, Wang D (2009) Serologic evidence of frequent human infection with WU and KI polyomaviruses. Emerg Infect Dis 15:1199–1205
101. Abed Y, Wang D, Boivin G (2007) WU poly- omavirus in children, Canada. Emerg Infect Dis 13:1939–1941
102. Norja P, Uballos I, Templeton K, Simmonds P (2007) No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. J Clin Virol 40:307–311
103. Han TH, Chung JY, Koo JW, Kim SW, Hwang ES (2007) WU polyomavirus in children with acute lower respiratory tract infections, South Korea. Emerg Infect Dis 13:1766–1768
104. Babakir-Mina M, Ciccozzi M, Perno CF, Ciotti M (2011) The novel KI, WU, MC polyomaviruses: possible human pathogens? New Microbiol 34:1–8
105. Dawood FS, Jain S, Finelli L, Shaw MW, Lind- strom S, Garten RJ et al (2009) Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 360:2605–2615
106. Garten RJ, Davis CT, Russell CA, Shu B, Lind- strom S, Balish A et al (2009) Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 325:197–201
107. Libster R, Bugna J, Coviello S, Hijano DR, Dunaiwsky M, Reynoso N et al (2010) Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in Argentina. N Engl J Med 362:45–55
108. Farias JA, Fernandez A, Monteverde E, Vidal N, Arias P, Montes MJ et al (2010) Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. Intensive Care Med 36(6):1015–1022 (Epub 2010 Mar 18)
99. Bishop JF, Murnane MP, Owen R (2009) Australia’s winter with the 2009 pandemic influenza A (H1N1) virus. N Engl J Med 361:2591–2594

100. Lister P, Reynolds F, Parslow R, Chan A, Cooper M, Plunkett A et al (2009) Swine-origin influenza virus H1N1, seasonal influenza virus, and critical illness in children. Lancet 374:605–607

101. Patel M, Dennis A, Flatter C, Khan Z (2010) Pandemic (H1N1) 2009 influenza. Br J Anaesth 104:128–142

102. Giannella M, Alonso M, Garcia de Viedma D, Lopez Roa P, Catalan P, Padilla B et al (2011) Prolonged viral shedding in pandemic influenza A (H1N1): clinical significance and viral load analysis in hospitalized patients. Clin Microbiol Infect 17:1160–1165

103. Bennett S, Gunson RN, MacLean A, Miller R, Carman WF (2011) The validation of a real-time RT-PCR assay which detects influenza A and types simultaneously for influenza A H1N1 (2009) and oseltamivir-resistant (H275Y) influenza A H1N1 (2009). J Virol Methods 171:86–90

104. Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L et al (2009) Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol 45:191–195

105. Faix DJ, Sherman SS, Waterman SH (2009) Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus H1N1, seasonal influenza virus, and critical illness in children. Lancet 374:605–607

106. Bennett S, Gunson RN, MacLean A, Miller R, Carman WF (2011) The validation of a real-time RT-PCR assay which detects influenza A and types simultaneously for influenza A H1N1 (2009) and oseltamivir-resistant (H275Y) influenza A H1N1 (2009). J Virol Methods 171:86–90

107. Meijer A, Jonges M, Abbink F, Ang W, van Beek J, van Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA et al. (2001) A newly discovered human pnuemovirus isolated from young children with respiratory tract disease. Nat Med 7:719–724

108. Sloots TP, Whiley DM, Lambert SB, Nissen MD (2008) Emerging respiratory agents: new viruses for old diseases? J Clin Virol 42:233–243

109. Bosis S, Esposito S, Osterhaus AD, Tremolati E, Begliatti E, Tagliabue C et al (2008) Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. J Clin Virol 42:286–290

110. Semple MG, Dankert HM, Ebrahim B, Correia JB, Booth JA, Stewart JP et al (2007) Severe respiratory syncytial virus bronchiolitis in infants is associated with reduced airway interferon gamma and substance P. PLoS One 2:e1038

111. Gerna G, Campanini G, Rognoni V, Marchi A, Rovida F, Piralla A et al (2008) Correlation of viral load as determined by real-time RT-PCR and clinical characteristics of respiratory syncytial virus lower respiratory tract infections in early infancy. J Clin Virol 41:45–48

112. McCullers JA (2006) Insights into the interaction between influenza virus and pneumococcus. Clin Microbiol Rev 19:571–582

113. McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM (2007) Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol 41:67–75

114. Peltola V, Heikkinen T, Ruuskanen O, Jartti T, Hovi T, Kilpi T et al (2011) Temporal association between rhinovirus circulation in the community and invasive pneumococcal disease in children. Pediatr Infect Dis J 30:456–461

115. Heikkinen T, Osterback R, Peltola V, Jartti T, Vainionpaa R (2008) Human metapneumovirus infections in children. Emerg Infect Dis 14:101–106

116. Aberle BH, Aberle SW, Redberger-Fritz M, Sandhofer MJ, Popow-Kraupp T (2010) Human metapneumovirus subgroup changes and seasonality during epidemics. Pediatr Infect Dis J 29:1016–1018

117. Mullins JA, Erdman DD, Weinberg GA, Edwards K, Hall CB, Walker FJ et al. (2004) Human metapneumovirus infection among children hospitalized with acute respiratory illness. Emerg Infect Dis 10:700–705

118. Chen X, Zhang ZY, Zhao Y, Liu EM, Zhao XD (2010) Acute lower respiratory tract infections by human metapneumovirus in children in Southwest China: a 2-year study. Pediatr Pulmonol 45:824–831

119. Manoha C, Bour JB, Pitoiset C, Darniot M, Aho S, Puthier P (2008) Rapid and sensitive detection of metapneumovirus in clinical specimens by indi-
rect fluorescence assay using a monoclonal antibody. J Med Virol 80:154–158

126. Williams J-V, Edwards K-M, Weinberg G-A, Griffin M-R, Hall C-B, Zhu Y et al (2010) Population Based Incidence of Human Metapneumovirus Infection among Hospitalized Children. J Infect Dis 201:1890–1898

127. Bosis S, Esposito S, Niesters HG, Crovari P, Osterhaus AD, Principi N (2005) Impact of human metapneumovirus in childhood: comparison with respiratory syncytial virus and influenza viruses. J Med Virol 75:101–104

128. Hackett S, Hill L, Patel J, Ratnaraja N, Ifeyinwa A, Farooqi M et al (2009) Clinical characteristics of paediatric H1N1 admissions in Birmingham, UK. Lancet 374:605

129. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J et al (2009) Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med 361:1935–1944