Newer respiratory virus infections: human metapneumovirus, avian influenza virus, and human coronaviruses

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Purpose of review

Recently, several previously unrecognized respiratory viral pathogens have been identified and several influenza A virus subtypes, previously known to infect poultry and wild birds, were transmitted to humans. Here we review the recent literature on these respiratory viruses.

Recent findings

Human metapneumovirus has now been detected worldwide, causing severe respiratory tract illnesses primarily in very young, elderly and immunocompromised individuals. Animal models and reverse genetic techniques were designed for human metapneumovirus, and the first vaccine candidates have been developed. Considerable genetic and antigenic diversity was observed for human metapneumovirus, but the implication of this diversity for vaccine development and virus epidemiology requires further study. Two previously unrecognized human coronaviruses were discovered in 2004 in The Netherlands and Hong Kong. Their clinical impact and epidemiology are largely unknown and warrant further investigation. Several influenza A virus subtypes were transmitted from birds to humans, and these viruses continue to constitute a pandemic threat. The clinical symptoms associated with these zoonotic transmissions range from mild respiratory illnesses and conjunctivitis to pneumonia and acute respiratory distress syndrome, sometimes resulting in death. More basic research into virus ecology and evolution and development of effective vaccines and antiviral strategies are required to limit the impact of influenza A virus zoonoses and the threat of an influenza pandemic.

Summary

Previously unknown and emerging respiratory viruses are an important threat to human health. Development of virus diagnostic tests, antiviral strategies, and vaccines for each of these pathogens is crucial to limit their impact.

Keywords

coronavirus, influenza A virus, metapneumovirus, respiratory disease

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Abbreviations

HCoV	human coronavirus
hMPV	human metapneumovirus
IAV	influenza A virus
RSV	respiratory syncytial virus
RTI	respiratory tract illness
SARS	severe acute respiratory syndrome

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Introduction

Acute respiratory tract infections are responsible for considerable morbidity and mortality in humans, and the costs attributable to acute respiratory tract illnesses (RTIs) are an important burden on national health care budgets [1]. A variety of viruses, bacteria and fungi are associated with RTIs [2], with most of the viruses belonging to the families of Paramyxoviridae, Orthomyxoviridae, Picornaviridae, Adenoviridae, and Coronaviridae. Since the beginning of this millennium, the Paramyxoviridae and the Coronaviridae virus families have been expanded, with the discovery of human metapneumovirus (hMPV), the coronavirus causing severe acute respiratory syndrome (SARS-CoV) and two previously unrecognized human coronaviruses identified in The Netherlands (HCoV-NL) and Hong Kong (HCoV-HKU1). In the past 2 years we have also become reacquainted with several influenza A virus (IAV) subtypes (family Orthomyxoviridae) that crossed the species barrier from birds to humans: subtype H7N7 in The Netherlands, H9N2 in Hong Kong and H7N2 in the USA in 2003, H5N1 in Southeast Asia in 2003 and 2004, and H7N3 in Canada and H10N7 in Egypt in 2004. Here we review the recent progress that has been made related to the fields of hMPV, avian IAV and HCoV infections.

Human metapneumovirus

Since its discovery in 2001 [3], hMPV has been detected in all continents of the world. Several recent studies, published after the first reviews on hMPV [4,5], have shed interesting new light on its clinical impact and epidemiology. Williams *et al.* [6••] estimated that hMPV was the likely causative agent in 12% of young children suffering from lower RTIs over a 25-year study period. In this and other studies, hMPV was found to be the second most detected pathogen in children suffering from acute

RTIs, topped only by respiratory syncytial virus (RSV) [6••,7•–9•]. It is difficult to determine the overall burden of disease caused by hMPV due to seasonality and geographical variation; while hMPV was detected in up to 57% of children suffering from RTIs during a single winter epidemic [10•], overall incidence estimates ranging from 5 to 12% are more realistic [4,6••,7•,11•,12•]. In young children, the clinical symptoms associated with hMPV infection are virtually indistinguishable from those caused by RSV, varying from mild upper RTI to severe pneumonia [4,5]. Very severe RTI was noted in children infected with both hMPV and RSV in some studies [13,14•] but not in others [6••,15•]. Since hMPV and RSV infections in many studies have been shown to occur in overlapping winter epidemics, co-infections with these two pathogens are not unlikely to occur, but their clinical importance requires more detailed studies. A controversy also exists with respect to the exacerbation of asthma upon hMPV infection, which was observed by some investigators [6••,7•,16–18], but not by others [19], and this remains an important question in hMPV research.

Information on the prevalence of hMPV and spectrum of disease associated with infection in people of other ages and other risk groups remain anecdotal. However, hMPV infections were detected in people with RTI of all ages, in the community or hospitalized, with most severe disease reported in frail elderly and immunocompromised individuals [4,7•,20,21]. Exacerbation of chronic obstructive pulmonary disease by hMPV was reported in five of 89 elderly individuals that were negative for other known pathogens [22]. The results of these recently published studies and those reviewed earlier [4,5] thus indicate that hMPV is an important pathogen in people of all ages. Further research is needed to obtain a better understanding of the clinical impact of hMPV infections in different populations, the full spectrum of hMPV-associated diseases and risk factors that may be associated with severe hMPV disease.

Although significant antigenic and genetic diversity has been noted for hMPV throughout the world, there is little evidence for antigenic drift over time [23•]. Using animal antisera, two serotypes have been identified, each of which can be further divided into two genetic lineages [23•,24•]. The full genome sequence has recently become available for each of the four genetic lineages of hMPV [25,26•,27]. The relevance of the antigenic variation for the induction of protective immunity in animal models and humans is still under debate [23•, 28•,29•]. It is of interest to note that reinfections with hMPV appear to occur frequently [30••], and that reinfections reported to date were caused by genetically distinct hMPVs [6••,31]. Recent evidence indicates that reinfections with genetically similar viruses can also occur (R. Spaete, personal communication). The genetic variation of hMPV is also important for the development and evaluation of diagnostic tests [32•], antiviral drugs and vaccines [28•,29•,33•]. Recently, Schildgen *et al.* [34] detected a nucleic acid sequence, distantly related to known hMPVs, in a nasopharyngeal aspirate collected from a child with acute asthma exacerbation. It remains to be confirmed whether this nucleic acid sequence indeed represents a novel hMPV lineage. If so, most of the currently available diagnostic tests for hMPV would need to be re-evaluated.

Several hMPV animal models have been developed for basic research and the evaluation of vaccine candidates and antiviral drugs. Hamsters, guinea pigs and ferrets were found to be most useful as small animal models, while cynomolgus macaques and African green monkeys represent good nonhuman primate models [28•,29•,33•, 35•]. The first experimental vaccines, using parainfluenza virus vector approaches, have been tested in rodents and primates and showed that the vectored vaccine viruses were attenuated, immunogenic, and protective [28•,33•, 36]. Such vaccines, targeting parainfluenza virus, hMPV and/or RSV at the same time may limit the impact of the most important RTIs of early childhood. Recently, reverse genetics approaches were developed for the generation of recombinant hMPV and avian pneumovirus, which will further facilitate future vaccine design [26•,37•,38]. These newly developed research tools will also be of great importance for future fundamental hMPV research.

Avian influenza A viruses

In 2003 and 2004, several incidents of human infections with avian IAVs were reported, caused by several different viral subtypes. Of greatest concern are the highly pathogenic avian IAVs of subtype H5N1 from Southeast Asia, which have caused extensive outbreaks in poultry since 1997, and resulted in fatal infection due to direct bird-to-human transmission [39–41]. In February 2003, H5N1 IAV was detected in two members of a Hong Kong family after a visit to Fujian province, China. While the father of the family died as a consequence of the infection, the 8-year-old son fully recovered [42•]. Shortly before these incidents, in December 2002, the H5N1 IAV was detected in a wide range of wild birds found dead in several nature parks in Hong Kong [43•]. This is unusual, as wild birds generally harbor only low pathogenic strains, and IAVs that are highly pathogenic to poultry are usually less pathogenic to wild fowl.

The geographically most extensive and still ongoing outbreak was first recognized in December 2003, when H5N1 IAV was identified as the causative agent in an outbreak of highly pathogenic avian influenza in poultry in the Republic of Korea [44]. Subsequently, outbreaks in

poultry caused by closely related IAVs were reported in Thailand, Viet Nam, Japan, China, Cambodia, Laos and Indonesia. In Viet Nam and Thailand, 44 confirmed cases of human infection with H5N1 IAV have been reported, of which 32 were fatal [45]. The prominent clinical features of some of these human cases have been described in detail, with fever, respiratory symptoms, diarrhea, lymphopenia, thrombocytopenia and abnormalities on chest radiography reported for most, if not all, patients [46••,47•,48]. Following initial observations that the 2004 H5N1 IAV also caused severe disease and death in tigers, leopards and pet cats in Thailand [49,50], it was shown experimentally that this virus can infect domestic cats, causes disease in these animals and can be transmitted between them [51•]. These observations and the observation that H5N1 IAV infection of pigs occurred in China [52] further highlight the threat these viruses pose to human and animal health.

The evolution of the H5N1 IAVs obtained during the outbreaks in Southeast Asia since 1997 has been investigated in detail [43•,53••,54••]. The HA and NA genes of the most recent IAVs appear to originate from those of 1997, and surveillance data indeed indicate that descendants of the H5N1 strains of 1997 have remained present in poultry or migratory ducks in China ever since. In contrast, the internal genes of recent H5N1 viruses were found to be more genetically diverse [53••], originating from a variety of sources by genetic reassortment. In agreement with the genetic analyses of the HA genes, the H5N1 IAVs isolated between 1997 and 2004 displayed clear differences in antigenic properties [43•,55••]. Moreover, it has become clear that the most recent H5N1 viruses, as compared with the 1997 viruses, have increased virulence for ducks and mice [43•,54••,55••] and possibly also for humans and other mammals. Overall, it is fair to conclude that the situation in Southeast Asia with respect to H5N1 IAVs is alarming, and warrants follow-up with appropriate pandemic planning scenarios and basic research (see below).

In The Netherlands, an outbreak of highly pathogenic avian influenza of subtype H7N7 hit the poultry industry in February 2003. During this outbreak the avian IAV was detected in 86 humans who handled affected poultry and three of their family members, despite the extensive control measures that were taken [56•,57•]. Of these 89 cases, 78 had conjunctivitis, five had conjunctivitis and influenza-like illness, two had influenza-like illness alone and four did not present with significant symptoms. While influenza-like illnesses were generally mild, one fatal case of acute respiratory distress syndrome occurred. Most IAV isolates obtained from humans, including probable secondary cases, were virtually identical to the chicken virus, but the fatal case virus displayed 14 amino acid substitutions, some of which may be associated with enhanced disease [56•]. After the outbreak, a serological survey conducted by the Dutch National Center for Infectious Disease Epidemiology indicated that an estimated 1000 people had been infected with the avian IAV. Antibodies to the H7 virus were detected in approximately 50% of the individuals handling infected poultry and 59% of poultry workers' family members [58]. While some of these data require confirmation, this study indicates that the previously reported number of human infections [56•,57•] was just the tip of the iceberg. This makes one wonder about the potential size of the iceberg with respect to other recent avian IAVs zoonoses, including those caused by H5N1 IAV.

More isolated cases of IAV zoonoses were also reported in the last year. An H9N2 IAV was isolated from a 5-year-old boy suffering from fever, cough and nasal discharge who was hospitalized for 2 days in Hong Kong in December 2003 [59]. The virus was genetically distinct from those detected in two hospitalized children in Hong Kong in 1999 [60]. Because H9N2 IAVs are highly prevalent in poultry in Eurasia, caused infection of humans and pigs in Asia, and are genetically and phenotypically diverse [61•], they continue to form a major point of concern. During an outbreak of highly pathogenic avian influenza in Canada in March 2004, an avian IAV of subtype H7N3 was isolated from two poultry workers involved in culling activities. Both poultry workers suffered from conjunctivitis and mild RTI or headache, and recovered completely [59,62]. On the same continent in New York, an H7N2 IAV was isolated from a man who suffered from RTI and was hospitalized in Westchester County in November 2003. While this virus was related to the IAV causing an outbreak in chickens in New Jersey, Maryland and Delaware in 2004, it is unclear how the man contracted the virus [59,63]. Finally, in Egypt in 2004, an avian IAV of subtype H10N7 was isolated from two young children suffering from fever and cough. Both children fully recovered [64].

As a consequence of the relatively frequent bird-to-human transmissions of avian IAVs in the last few years, 'pandemic preparedness' has become a key issue [65••]. The WHO Global Agenda for Influenza Surveillance and Control that was adopted in 2002 has set four main objectives: strengthening surveillance; improving knowledge of the disease burden; increasing vaccine usage; and accelerating pandemic preparedness [66•]. The influenza research community has acted in accordance with this agenda. Candidate vaccines have been prepared for avian viruses of subtypes H9N2 [67•,68•] and H5N1 [69•]. However, there are several problems related to such vaccines, making it unlikely they could become available for human use in the short term (reviewed in [65••,70,71•]). Drugs such as neuraminidase inhibitors

are another tool to protect against disease from IAV infection. Recent evidence suggests, however, that resistance to such drugs could become a problem when they are used on a large scale [72•]. Increased knowledge on emerging avian IAVs in the human population could aid in the early recognition of pandemic threats, and will be of key importance in the years to come. While the molecular determinants for binding of IAV hemagglutinin to avian and human cellular receptors are now relatively well known, and the structural basis for these determinants has recently been resolved for earlier pandemic strains [73•,74•], the contribution of other viral genes and their products in determining virus transmission and host range remains less clear. Understanding the molecular determinants of IAV pathogenicity and, more importantly, transmission and host range should thus continue to be a major research objective.

Newly discovered human coronaviruses

The SARS outbreak that affected 26 countries between November 2002 and July 2003 caused 774 human deaths of 8098 SARS cases. The outbreak was caused by a previously unknown coronavirus, SARS-CoV, which probably originated from an animal reservoir. Reviews of SARS describing etiology, transmission, clinical presentation, diagnosis, treatment, and vaccines were published recently in this journal [75–77], and will thus not be discussed again here. However, two additional previously unrecognized coronaviruses affecting humans were identified in 2004. In The Netherlands, two independent research teams isolated a previously unknown coronavirus from patients suffering from RTI, named HCoV-NL or HCoV-NL63 [78•,79•]. The virus was isolated in tertiary monkey kidney cells and propagated in Vero cells. Complete sequencing of the viral genome revealed that HCoV-NL was most closely related to the human coronavirus 229E and the porcine epidemic diarrhea virus, which are both group-1 coronaviruses. The virus was detected in eight of 493 individuals with RTI in Amsterdam, and in four of 139 individuals with RTI of unknown etiology in Rotterdam. The symptoms of the patients from whom the virus was isolated ranged from mild RTI to severe pneumonia. While most HCoV-NL-infected patients in the Rotterdam study were young children with underlying disease, the patients in the Amsterdam study were otherwise healthy and their ages ranged from 4 months to 67 years.

The third newly discovered coronavirus was identified in Hong Kong in a nasopharyngeal aspirate sample collected from a 71-year-old man suffering from pneumonia [80]. The virus, HCoV-HKU1 was identified by reverse-transcriptase polymerase chain reaction, using primers specific for the coronavirus family. Despite the fact that the virus could not be isolated in a wide variety of cell cultures, Woo *et al*. were able to determine the nucleotide sequence of the full viral genome. HCoV-HKU1 was identified as a group-2 coronavirus, only distantly related to known group-2 coronaviruses, including HCoV-OC43. Upon testing of 400 nasopharyngeal aspirate samples collected from individuals with RTI during the SARS outbreak, HCoV-HKU1 was detected in one additional patient: a 35-year-old woman suffering from pneumonia [80].

The detailed investigation of the clinical impact and epidemiology of HCoV-NL and HCoV-HKU1 is an important new research area.

Conclusion

Respiratory viral pathogens, old and new, continue to be an important threat to human health. Four previously unrecognized viruses associated with respiratory disease in humans were identified in the last 3 years, while several known avian IAVs were transmitted from birds to humans, causing serious concern of an influenza pandemic. State-of-the-art virus diagnostic techniques remain crucial for the rapid identification of known and unknown pathogens. For each of the viruses discussed here, our understanding of virus epidemiology, evolution, pathogenesis, transmission, clinical presentation and host defense against infection are incomplete. It will be essential to further increase this knowledge to control the impact of these viral pathogens on human health, for instance through development of effective intervention strategies.

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