An Overview of Respiratory Viruses Including an Update on Avian Influenza

Introduction: In the 2005-06 survey of General Practice Activity in Australia (Australian GP Statistics and Classification Centre), respiratory problems constituted the largest group of problems managed by General Practitioners at 14.1% of all problems. Within the respiratory group of problems, upper respiratory tract infections (URTIs) were the largest sub-group.

Viral pathogens are the most common causes of respiratory tract infections. These agents are responsible for considerable morbidity and in some cases mortality, resulting in decreased economic productivity and cause a great demand on medical services. In addition, the inappropriate treatment of viral infections with antibiotics is believed to contribute to the alarming rise of antibiotic resistant bacteria. However, knowledge of these agents remains sketchy to many practitioners, probably because diagnostic tests until recent years have been cumbersome and slow. Developments in nucleic acid based laboratory tests over the last decade have changed this situation and it is now possible to identify many viral pathogens within a clinically useful timeframe. These tests are readily available through Clinipath Pathology.

In the new millennium, there have been a number of significant developments involving respiratory viral agents. Several new agents have been discovered and the world population has been seriously threatened by epidemics and outbreaks – for example the SARS coronavirus and the highly pathogenic avian influenza virus (HPAI) H5N1. There is, in addition, an increasing understanding of the role of common respiratory viral infection in exacerbating both asthma and chronic bronchitis.

The aim of this article is to provide an overview of the commonly encountered respiratory viral agents and the current status of H5N1 avian influenza virus.

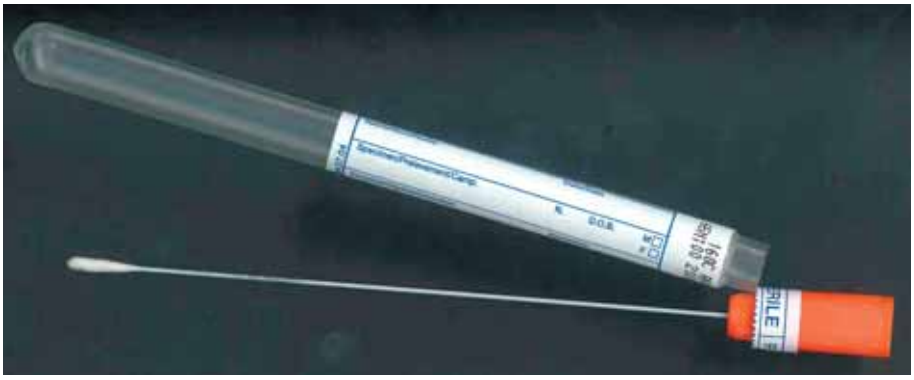
When we speak of respiratory viruses, we typically mean rhinovirus, coronavirus, adenovirus, parainfluenzavirus, influenzavirus, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV). Other viruses, however, may also cause disease at different levels of the respiratory tract and these include Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), enteroviruses and measles virus. This discussion will focus largely on the former group. Bacterial pathogens are not considered here.

A number of disease entities of the respiratory tract may be caused by respiratory viruses and they are:

- rhinitis
- sinusitis
- otitis media
- pharyngitis
- laryngitis
- epiglottitis
- laryngotracheitis
- laryngotracheobronchitis
- bronchitis
- bronchiolitis
- pneumonia/pneumonitis

For each of these entities, a number of different respiratory viruses may be responsible; the number of potential causes may be broad or narrow depending on the entity. Pharyngitis, for example, may be caused by rhinovirus, coronavirus, adenovirus, parainfluenzavirus, influenzavirus, EBV, HSV and enteroviruses, whereas laryngotracheitis is more likely to be caused by parainfluenza type 1 and other causes are less common. Age and other factors may also influence the manifestations of infection with a particular agent.

Rhinoviruses are the most frequent causes of the common cold. There are over 100 different serotypes of rhinoviruses; the host's immune response in general protects against reinfection with the same serotype for many years, but colds are frequent because of infection with different serotypes. The main features of a rhinovirus cold are rhinorrhoea and blocked nose but mild pharyngitis is also common. Fever is not usually present. Involvement of the mucosa of the paranasal sinuses is now accepted as part of the pathology of rhinovirus colds in the majority of cases. Rhinovirus has been isolated from the middle ear of some cases of otitis media. This virus is a cause of half or more attacks of asthma in both children and adults and is implicated as a cause of exacerbations of bronchitis in patients with COPD. In temperate climates, rhinovirus activity in the population is present throughout the year with increased activity in autumn and spring. Data from Perth tend to show irregular seasonal variation from year to year with the lowest frequency of isolation being in January-February.



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Coronaviruses are important pathogens of a number of animal species including birds. Until recently, two strains of coronavirus (HuCoV-OC43 & HuCoV-229E) were known to infect humans in whom they are the second most frequent causes of the common cold. The signs and symptoms of coronavirus colds are very similar to those caused by rhinoviruses although the duration of illness is shorter. Coronaviruses have been isolated from infants with pneumonia and from military recruits with pneumonia. There is an association with precipitation of wheezing in asthmatic children and exacerbation of bronchitic symptoms in adults with COPD. Coronavirus infections are more severe in the elderly.

In this new century, three new coronaviruses have been discovered. The first of these, HuCoV-NL, has caused a small number of infections in The Netherlands, ranging from mild respiratory tract infection to pneumonia. The second, HuCoV-KKU1, has been isolated from two patients in Hong Kong with pneumonia. The major new coronavirus discovery, however, has been the SARS Coronavirus (SARS-CoV), the cause of the Severe Acute Respiratory Syndrome epidemic in 2002-03. This epidemic illustrates the consequences of a virulent and contagious agent crossing the species barrier from animal to man, but also the effectiveness of a coordinated response in identifying and controlling a novel infectious agent. In early February of 2003, the Chinese health authorities reported to the World Health Organisation 305 cases of severe respiratory infection that had occurred in Guangdong Province since November of the previous year. Although there had been 5 deaths, the epidemic was thought to be under control and was attributed to Chlamydophila pneumoniae. At the end of February, however, a similar illness was recognised in Hanoi and in March was seen in Hong Kong. In both of these places, the illness occurred in hospital staff. At this time the WHO released a case definition of SARS and issued a Global Alert. An International Multicentre SARS Research Project was established linking eleven laboratories in ten countries. The infectivity of the agent and the role of air travel in its spread were sharply illustrated by the Metropole Hotel incident. On the night of February 21st 2003, a Chinese professor who had been treating SARS cases in Guangdong Province stayed overnight in the Metropole Hotel in Hong Kong. He infected 12 other guests and after becoming unwell and hospitalised, infected 4 health care workers, 2 family members and then died. By March 26, the 12 infected hotel guests had gone on to infect 247 others in Hong Kong, Hanoi, Singapore and Canada, 192 of whom were health care workers. Two of the hotel guests and eight of whom they infected, died. The role of travel, particularly air travel, in spreading the disease was quickly recognised and controls placed on travellers from SARS affected areas helped to limit its spread. One interesting strategy to detect febrile travellers was the use of thermal body imaging at several international airports. The international effort to identify the cause of SARS bore fruit with the laboratory isolation of the putative agent in Hong Kong on March 22. This was identified as a novel coronavirus on April 16 and 2 weeks later its complete genetic sequence had been determined. This was found to be nearly identical to viruses isolated from exotic animals sold for consumption in Chinese street markets, specifically the masked palm civet cat and the racoon-dog. By the time the epidemic had been brought under control at the end of July 2003, there had been 8422 cases identified and 916 deaths. Mortality exceeded 50% in those over 65 and in those debilitated with chronic illnesses.

Another group of viruses causing respiratory infections are the **adenoviruses**, of which there are over 50 known serotypes. Only about half of these are confirmed to be responsible for disease in humans. Other than respiratory infections, particular serotypes cause diarrhoea, haemorrhagic cystitis in children, meningoencephalitis and keratoconjunctivitis. Several forms of respiratory disease are caused by adenoviruses and these depend on the serotype involved and the age of the patient. Infants can develop severe bronchiolitis and pneumonia and children may suffer from pharyngitis and tracheitis. Pharyngoconjunctival fever is another manifestation seen in children. The common cold, however, is the most likely outcome of respiratory adenovirus infection. Fever, cough, sore throat and rhinorrhoea are usually present and there may be patchy infiltrates on chest x-ray. Not taking particular serotypes into account, adenovirus activity in WA

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can differ from year to year. In some years it may be distributed without pattern across the months but in other years there is greater activity seen in the second half of the year including the summer months.

Respiratory syncytial virus (RSV) is the major cause of bronchiolitis and pneumonia in infants under 1 year old. RSV infection in infants begins with fever, rhinitis and cough and within several days, a high proportion will develop bronchiolitis and/or pneumonia. The manifestations of infant bronchiolitis are explained by the obstruction of small airways by sloughed necrotic epithelial cells and mucus. The cough worsens and becomes productive and signs of small airways obstruction develop in the form of wheezing and crackles. The obstruction is both inspiratory and expiratory; because the latter is more marked air trapping and hyperinflation of the chest results. Respiratory distress results in hospital admission in a substantial proportion of infants and hypoxaemia is frequently present in this group. Half of those who experience RSV bronchiolitis in infancy will experience recurrent wheezing episodes as older children. Immunity to RSV is short lived and reinfections in children and adults are common. Reinfections are rarely as severe as the initial infection and present with cold symptoms, but these are more severe than in colds produced by rhinovirus, coronavirus or adenovirus. Sinus and middle ear involvement is more common. Lower respiratory involvement is frequent and includes tracheobronchitis or pneumonia with CXR infiltrates. The virus should be considered when otherwise healthy adults present with prolonged cough. In the elderly, particularly those who are institutionalised or suffer from chronic cardiopulmonary conditions, RSV is a cause of significant morbidity and mortality. In Perth, RSV activity is very sharply defined in terms of its seasonal distribution. It is uncommon during the hot months; cases begin to appear in April and from May-June climb steeply to a high peak in July and then fall away to almost nothing in October-November.

Human metapneumovirus (hMPV), discovered in 2001, is related to RSV and produces similar illness. The peak of severe illness in infants occurs a little later, at 3-6 months of age compared to 2 months with RSV. In Perth, its peak incidence occurs about 3 months later than that of RSV, being highest in October.

Parainfluenzaviruses consist of types 1, 2, 3, 4a and 4b. The latter two are rarely isolated. Types 1, 2 & 3 are causes of upper or lower respiratory tract infection. Type 1 (and to a lesser extent types 2 & 3) is the main cause of two conditions in the croup spectrum: croup (laryngotracheitis) and spasmodic croup. Laryngotracheitis occurs following infection of the supraglottic, glottic and subglottic mucosa which becomes swollen to the extent of causing partial obstruction. Children present with coryza, fever, hoarse voice, barking cough and inspiratory stridor. With increased subglottic narrowing there is marked sternal recession upon inspiration and affected children are quite distressed. Spasmodic croup is a less severe syndrome occurring at night-time and consisting of prolonged coughing and stridor. Allergic factors are thought to have a role. The parainfluenzaviruses also cause URTIs in which otitis media is frequently present. They are also becoming increasingly recognised as causes of serious lower respiratory tract infections in the elderly and immunocompromised.

In terms of impact on population health, the **influenza viruses** are the most important of the respiratory viruses. The influenza viruses consist of three serotypes: A, B and C. Type A and to a lesser extent Type B produce regional outbreaks and epidemics in the human population. Disease occurs on a spectrum ranging from cold-like symptoms to severe life threatening pneumonia with systemic features.

The influenza viruses are characterised by surface proteins which are visible by electron microscopy on the surface of the virus as a layer of short projections. Two important surface proteins are haemagglutinin (H) and neuraminidase (N). These surface proteins are virulence factors contributing to the pathogenicity of the virus and in addition their antigenic uniqueness is used for classifying different strains of the virus into subtypes.

There are 15 different H subtypes and 9 different N subtypes allowing for 135 potential different viral strains. Only 3 haemagglutinins (H1, H2, H3) and 2 neuraminidases (N1, N2) are found in influenza A viruses ordinarily infecting humans. The two major subtypes of influenza virus circulating in the world's human population at the present time are designated H1N1 and H3N2, the

numbers indicating that the two viruses have distinct surface proteins. Individuals and consequently populations will develop antibodies to the haemagglutinins and neuraminidases of the viruses to which they are exposed.

Influenza A viruses continue to cause outbreaks of infection because they have the ability to change the antigenic properties of their surface proteins, a process called antigenic variation. The result of antigenic variation is an influenza virus to which the population has reduced immunity.

There are two types of antigenic variation: the frequent but minor changes called antigenic drift and the infrequent, major change called antigenic shift. Antigenic drift results from minor mutations in the genes encoding the surface proteins and is responsible for the yearly outbreaks of influenza experienced by the population. Antigenic shift results from exchange of genetic material between a human influenza virus and an animal influenza virus (genetic reassortment) resulting in quite distinct surface protein(s) and change in nomenclature (eg. H2N2 becomes H3N2). Antigenic shift is responsible for the world pandemics of influenza occurring several times in a century.

Animals clearly have an important role in the phenomenon of antigenic shift. It is believed that swine are the hosts for coinfection with human and avian influenza viruses allowing genetic reassortment to take place.

Numerous subtypes of influenza A viruses specifically infect animals including birds, pigs and horses. Wild birds are the main reservoir for all influenza A subtypes and the source of infections in other animals. Wild birds are frequently asymptomatically infected but farmed birds are susceptible to serious disease with the virus. Epidemics of influenza cause significant mortality in poultry flocks worldwide from time to time. It is uncommon for influenza A subtypes to cross species barriers. However, human subtypes have been known to cross into animals

Table 1.

Countries in which H5N1 has been isolated from birds since 2003.

(Distinction is not made between isolated cases in wild birds and outbreaks in farmed birds)

Afghanistan	Albania	Austria
Azerbaijan	Bangladesh	Bosnia and Herzegovina
Bulgaria	Burkina Faso	Burma
Cambodia	Cameroon	China
Côte d'Ivoire	Croatia	Czech Republic
Denmark	Djibouti	Egypt
France	Georgia	Germany
Greece	Hong Kong	Hungary
India	Indonesia	Iran
Israel	Italy	Japan
Jordan	Kazakhstan	S. Korea
Kuwait	Laos	Malaysia
Mongolia	Niger	Nigeria
Palestinian Territories	Pakistan	Poland
Romania	Russia	Saudi Arabia
Serbia and Montenegro	Slovakia	Slovenia
Spain	Sudan	Sweden
Switzerland	Thailand	Turkey
Ukraine	United Kingdom	Vietnam

and strains from birds and pigs have infected humans. The first recorded human infections with an animal subtype occurred in 1997 when 18 individuals were infected with the H5N1 strain of avian influenza in Hong Kong. Six of these cases were fatal. Other instances of human infection with avian subtypes have occurred since that time:

1998-99: Sporadic mild H9N2 human infections in China and Hong Kong. One more case in Hong Kong in 2003.

2002: One case of subclinical H7N2 human infection in Shenandoah Valley, USA. Another in New York in 2003.

2003: Two cases of highly pathogenic H5N1 human infections in Hong Kong, acquired in mainland China; one died.

2003: An outbreak of H7N7 avian influenza originating in Holland caused mild infection in 89 people, mostly poultry workers. Most of these consisted of conjunctivitis, several had influenza and there was one death.

2004: Cases of conjunctivitis in poultry workers in Canada, caused by H7N3.

Of greatest concern has been the re-emergence of human infections caused by the highly pathogenic avian H5N1 subtype. In 2003, this virus was reported causing epidemics in poultry in several Asian countries and in early 2004, human cases began to be reported. Since that time infections in birds have been reported from 57 nations (Table 1) and human infections have been reported from 12 nations (Table 2).

Unlike the human strains of influenza virus which cause predominantly respiratory symptoms, the highly pathogenic H5N1 subtype has the ability to infect a number of cell types, and human cases have been characterised

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by multiorgan involvement. The disease in humans produces mortality greater than 50%.

Over the course of this epidemic, the majority of cases of human H5N1 have occurred in people in close contact with farmed poultry. There have been, however, several cases of human to human spread occurring in Thailand and Indonesia. The great concern at present is that H5N1 may undergo genetic reassortment with a human strain of the virus, resulting in a highly virulent strain with efficient human to human transmission. Such an outcome would pose a real threat of a worldwide human pandemic of high mortality.

Diagnostic tests are available locally for H5N1 infection. However, these are only performed on patients where there is a probability of exposure. At present the criteria for testing are:

A person who develops an influenza-like illness who, in the seven days prior to onset of illness:

1. Has had direct contact with sick chickens or carcasses in an area with current HPAI activity OR
2. Is a health care worker directly involved in the care of human avian influenza cases OR
3. Is a family member or other close contacts of a known human case OR
4. Is a laboratory worker who has been working in laboratory with HPAI

Table 2.

Countries in which human cases of H5N1 infection have been documented since 2003.

Country	Cases	Deaths
Azerbaijan	8	5
Cambodia	7	7
China	24	15
Djibouti	1	0
Egypt	34	14
Indonesia	81	63
Iraq	3	2
Laos	2	2
Nigeria	1	1
Thailand	25	17
Turkey	12	4
Vietnam	93	42
Total	**291**	**172**

Tourists travelling through areas of HPAI activity are not considered to be at risk unless there have been special circumstances involving exposure. The Clinical Microbiologist should always be alerted before arrangements are made to collect specimens.

Appropriate specimens for diagnosis are anterior nasal swabs, nasopharyngeal swabs or throat swabs. If these are submitted in viral transport medium, they can be used for both PCR testing and viral culture. An acute phase serum sample should also be collected.

In terms of diagnosing infections with the other respiratory viruses, PCR testing is available for all of these and is performed on respiratory tract secretions, nasopharyngeal swabs, nasopharyngeal aspirates or throat swabs. The earlier in the course of the infection the specimens are collected, the more likely they are to produce positive results. Serological testing is also available for antibodies to most of the respiratory viruses.

In general, antibodies may not be detectable in the first week of the illness. Collection of a second specimen in the second week provides the opportunity to demonstrate seroconversion or increases in the antibody level.

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