NOVEL AND
RE-EMERGING
RESPIRATORY VIRAL
DISEASES
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NOVEL AND RE-EMERGING RESPIRATORY VIRAL DISEASES
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Editors: Gregory Bock (Organizer) and Jamie Goode

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Chair’s introduction

Robert G. Webster

Emerging and re-emerging infectious diseases are part of the natural history of humankind, for there has always been a struggle between microbes and humans. A considerable part of the human genome is concerned directly or indirectly with strategies to combat infectious diseases. Humans have continued their global dominance and in the past century have used scientific knowledge to reduce the impact of novel disease agents. The ever-increasing human population expansion and factors such as land use, water use and energy use needed to support the burgeoning human population, has resulted in production of animals on megafarms in close proximity to wild animals and birds. The export of intensive farming practices to the developing world, for example chicken and pig raising, has not always been accompanied by the best practices for ensuring bio-security and disease prevention in those operations. Thus intensive poultry and pig raising, without adequate separation from free-flying birds and water treatment, is a recipe for disaster. The increasing number of outbreaks of lethal H5 and H7 influenza, in domestic poultry, globally attests to these assertions.

The emergence of novel infectious diseases is a continuing process with multiple novel agents emerging in the past decade. While many of these agents caused transitory disease outbreaks—Nepah virus from bats to pigs and people in Malaysia, and Hendra virus from bats to horses and people in Australia—that were rapidly identified and stamped out, others became endemic in humans and in domestic animal species. Notable examples are human immunodeficiency virus (HIV) (African primates to humans) and West Nile virus (introduction to the Americas from Europe and spread through mosquitoes to wild birds, domestic mammals and humans).

Two recent examples of emerging infectious disease agents are severe acute respiratory syndrome (SARS) and highly pathogenic H5N1 avian influenza (‘bird flu’). These two disease agents are the main topics for this meeting. Both of these diseases are caused by RNA viruses of zoonotic origin; SARS by a novel coronavirus from bats via civet cats in live animal markets (‘wet markets’) to humans, and H5N1 bird flu by a type A orthomyxovirus from wild aquatic birds via
domestic poultry to humans. Both of these emerging infectious diseases were ‘man
made’ in the sense that increased affluence of humans in the region increased the
demand for protein in the diet. Intensified animal raising and the demand for
exotic wild animal meat permitted these viruses to initially spread to humans in
Hong Kong and Southern China through wet markets. The actual precursor
viruses of neither SARS nor H5N1 bird influenza have been identified, but their
closest genetic relatives were detected in animals and poultry in wet markets at the
time they initially spread to humans.

Southeast Asia has been described as the epicentre for the emergence of pan-
demic influenza viruses, including the Asian H2N2 influenza of 1957, the Hong
Kong H3N2 virus of 1968, as well as the re-emerging H1N1 Russian influenza
virus of 1977. Both the H5N1 highly pathogenic avian influenza virus and the
SARS coronavirus emerged in this region of the world. While culling of all domes-
tic poultry in Hong Kong in 1997 successfully stamped out the initial genotype of
H5N1, the virus re-emerged from apparently healthy ducks and geese in the region
and spread to multiple countries in Southeast Asia including Vietnam, Cambodia,
Laos, Indonesia, Japan and South Korea. The virus was largely restricted to the
Southeast Asia region until 2005. The dramatic spread of the virus in mid-2005
occurred after H5N1 infected Bar-headed geese and other wild water fowl in
Qinghai Lake in Western China. After that event, the virus spread rapidly through
the Indian subcontinent, the African continent and Europe. The role of migratory
birds seems probable. While the highly pathogenic H5N1 virus continues to spread
throughout Eurasia it has, to date, not spread to the Americas despite the overlap
of migrating birds in Alaska.

Both SARS and H5N1 bird flu are similar in being poorly transmissible in
humans. During the SARS outbreak, this virus infected 8096 persons globally with
774 deaths (9.6%), while H5N1 bird flu has infected over 300 humans with 60%
lethality. The poor transmissibility of SARS led to the control of this virus by
conventional biosecurity and quarantine. While SARS is under control, H5N1 bird
flu is not. H5N1 appeared in Hong Kong a decade ago: it has now spread to over
60 countries in Eurasia and has evolved into at least four antigenically distinct
clades. Although H5N1 has not acquired consistent human-to-human transmis-
sion the possibility exists that we may be witnessing the evolution of a human
influenza pandemic in real time.

Dr Yee-Joo Tan from The Institute of Molecular and Cell Biology, Proteos,
Singapore, who participated in the battle against SARS in Singapore, proposed the
topic of emerging and re-emerging respiratory viruses as the subject for the present
meeting. Both the topic and the site for the meeting were most appropriate.
Although the economic impact of SARS turned out to be relatively short term (due
to rapid acquisition of scientific knowledge and control strategies) the initial impact
on service exports in Singapore and Hong Kong, especially on tourism, was par-
particularly severe. If SARS had not been controlled so expediently, the economic impact would have been much worse.

The lessons from SARS are certainly applicable to the expanding problem of H5N1 bird flu and to future emerging infectious diseases. The successful containment of SARS and the lessons learned from that successful programme are important to be considered in the face of a possibly emerging influenza pandemic in humans. However, we must keep in mind that the transmissibility of influenza is likely to be very different from that of the SARS coronavirus.
Identification and characterization of novel viruses

Larry J. Anderson and Suxiang Tong

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Abstract. Although much has been learned about the agents and the clinical and epidemiological features of acute respiratory illness (ARI), much still remains unknown. Among children in the USA, the agent of 25–50% of cases of ARI remains unknown and among adults the agent remains unknown for about 50% of ARI cases. Roadblocks to detecting the etiological agent include quality of specimens, sensitivity and specificity of assays, and probably presence of as yet unknown pathogens. For example, since the year 2000, five new viral agents of ARI have been identified (human metapneumovirus [hMPV], SARS CoV, two human coronaviruses [NL63 and HKU1] and a new parvovirus [human bocavirus]). A variety of methods have been used to try to detect novel viral pathogens and include classic techniques such as tissue culture isolation, antigen detection assays and electron microscopy, and molecular methods designed specifically to detect novel pathogens. Examples of different successful methods to detect novel pathogens include those used to identify the hepatitis C virus, human herpes virus 8, Sin Nombre virus, and SARS coronavirus. At CDC, we have developed several molecular methods to identify new pathogens including pan viral family PCR assays that can detect any member of a given family of viruses. To date, we have developed pan viral family (or genera) PCR assays for 11 viral families. The improving methods to discover new viruses are likely to present investigators with the challenge to determine if and what disease an increasing number of novel viruses might cause. Koch's postulates provide guidelines for establishing a causal relationship between a pathogen and disease and include establishing an epidemiologic link between the pathogen and disease and then showing a causal relationship, most often through animal inoculation studies. The success of new molecular tools for pathogen discovery highlight the need for more efficient ways to determine what disease might be associated with the infection.

2008 Novel and re-emerging respiratory viral diseases. Wiley, Chichester (Novartis Foundation Symposium 290) p 4–16

Acute respiratory illnesses (ARI) include the common cold, bronchitis, bronchiolitis, croup, sore throat and pneumonia, and are the most common illnesses of humans. They are a major cause of morbidity and mortality world-wide. It is estimated that globally ~2 million deaths/year occur in children <5 years old from

1Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.
ARI (Williams et al 2002). In adults in the USA, there are an estimated 1 million cases of community acquired pneumonia each year and pneumonia is among the 10 most common causes of death. As noted in Table 1, there is wide variety of viral and bacterial pathogens commonly associated with ARI. Influenza viruses are the most important cause of serious viral ARI and respiratory syncytial virus (RSV) probably is the second most important viral respiratory pathogen. Influenza has most often been considered an important pathogen in adults but can also be a significant cause of ARI in children (Weinberg et al 2004). RSV is most often associated with serious disease in the infant and young child but also causes serious ARI disease throughout life (Falsey et al 2005). Our understanding of viral ARI is changing because of the discovery of novel viruses and the availability of better diagnostic assays. Five novel respiratory viruses including human metapneumovirus (van den Hoogen et al 2001), SARS coronavirus (CoV) (Drosten et al 2003, Ksiazek et al 2003, Peiris et al 2003), two novel human coronaviruses, NL63 and HKU1 (Fouchier et al 2004, van der Hoek et al 2004, Woo et al 2005), and human bocavirus (Allander et al 2005) have been discovered since 2000. Improved diagnostics, especially sensitive polymerase chain reaction (PCR) assays, have also made it possible to consistently identify difficult to detect viruses such as rhinoviruses, and it is becoming increasingly clear that rhinoviruses have been under appreciated as serious respiratory pathogens (Miller et al 2007, Falsey et al 2002). Even with the discovery of novel viruses and improved diagnostics, there remain a significant number of ARIIs for which the aetiology remains unknown. In adults, up to 50% of hospitalized patients with lower respiratory tract illnesses (LRIs) have no aetiological agent detected. Between 25% and 50% of children hospitalized with LRI have no aetiological agent detected. Some of these undiagnosed

<table>
<thead>
<tr>
<th>TABLE 1 Pathogens associated with acute respiratory illness (ARI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral agents</strong></td>
</tr>
<tr>
<td>Adenoviruses</td>
</tr>
<tr>
<td>Coronaviruses including SARS CoV</td>
</tr>
<tr>
<td>Enteroviruses</td>
</tr>
<tr>
<td>Human bocavirus</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
</tr>
<tr>
<td>Human parainfluenza viruses 1–4</td>
</tr>
<tr>
<td>Influenza virus A and B (Flu A and B)</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus (RSV)</td>
</tr>
<tr>
<td>Rhinoviruses</td>
</tr>
<tr>
<td><strong>Bacterial agents</strong></td>
</tr>
<tr>
<td><em>S. pneumonia</em>, <em>H. influenza</em>, <em>M. pneumonia</em>, <em>Chlamydia</em>, etc.</td>
</tr>
<tr>
<td>Gram negatives, <em>M. tuberculosis</em>, <em>Legionella</em> species, other</td>
</tr>
<tr>
<td><strong>Unknown pathogens</strong></td>
</tr>
</tbody>
</table>
illnesses may be caused by as yet unknown pathogens and others by known pathogens but existing methods are either not sufficiently specific or not sufficiently sensitive to confirm the diagnosis. For example, detecting *Streptococcus pneumoniae* infection in the upper respiratory tract is not sufficiently specific to confirm it as the aetiology of pneumonia (Butler et al 2003). RSV infection is difficult to diagnose in adults because the assays traditionally used to detect infection are not sufficiently sensitive. In a study by Falsey et al (Table 2), most RSV infections (about 75%), diagnosed serologically with acute- and convalescent-phase serum specimens, are detected with a sensitive, nested PCR assay but only about one-third by virus isolation (Falsey et al 2002). Sensitive PCR assays can also improve our ability to detect viral respiratory infections in children as illustrated in a study by Weinberg et al (2004) (Fig. 1).

Novel viruses have been detected in illnesses of unknown aetiology, including ARIs, through various combinations of traditional and newer molecular methods.

### TABLE 2 Detection of RSV in 1112 elderly patients

<table>
<thead>
<tr>
<th>Serology</th>
<th>Number</th>
<th>PCR+*</th>
<th>Isolation +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>104 (9.4%)</td>
<td>74 (6.7%)</td>
<td>37 (3.3%)</td>
</tr>
<tr>
<td>Negative</td>
<td>1008 (90.6%)</td>
<td>13 (1.1%)**</td>
<td>6 (0.5%)</td>
</tr>
</tbody>
</table>

* Nested PCR assay.
** 6/13 were isolation +.

Note: 117+ (serology detected 89%; PCR 74%; isolation 37%).
Adapted from Falsey et al (2002).

![Graph showing detection of respiratory pathogens: sensitive PCR versus isolation or antigen detection](image)

**FIG. 1.** Detection of respiratory pathogens: sensitive PCR versus isolation of antigen detection. Adapted from Weinberg et al (2004).