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Influenza Report 2006

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Preface

Thirty years ago, infectious diseases were seemingly on the decline. Tuberculosis was defeated, small pox was about to be eradicated, sexually transmissible diseases could easily be treated, and other scourges of mankind, such as malaria, were expected to disappear one day. Some experts hilariously announced that we would soon be able to close the book of infectious diseases once and for all. Of course, that was before the beginning of the AIDS pandemic in 1981, and before the discovery of the hepatitis C virus, as well as many other viruses capable of causing severe disease in humans.

Human memory is permeable and porous. A quick look at medical history would have sufficed to understand that infectious diseases have accompanied humans ever since they opted for a sedentary lifestyle. While we are today better prepared to prevent and fight off infectious diseases, we are nonetheless condemned to coexist with them. In a world with an increasing potential for the rapid spread of pathogens – overcrowded cities, high mobility – the role of efficient infectious disease task forces can therefore not be overestimated.

In the wake of HIV, hepatitis C, drug-resistant tuberculosis, and SARS, another devastating influenza pandemic may be the next global health threat that six and a half billion people will have to face. An avian influenza strain, H5N1, has recently caused multiple outbreaks in poultry on three continents and has infected nearly 200 persons, killing more than half of them. The timing and the magnitude of the next pandemic is all but certain, but it is wise to be prepared.

Influenza Report provides a comprehensive overview of human and avian influenza. The book is freely available on the Internet and the second edition is scheduled to be published before the end of the year. *Influenza Report* may be translated into other languages without incurring a license fee (see details on the website). The philosophy which governs the publication of the report has recently been published at www.freemedicalinformation.com.

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Paris, Hamburg, Tygerberg – 24 March 2006

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Content

Chapter 1: Influenza 2006.....	17
Global Impact	17
Epidemics and Pandemics	17
1918.....	21
1957.....	22
1968.....	22
Current Situation	22
Individual Impact.....	23
The Virus	24
Requirements for Success	24
Virology	25
Natural Reservoir + Survival.....	26
Transmission	27
H5N1: Making Progress.....	28
Individual Management.....	29
Epidemic Prophylaxis	30
Epidemic Treatment	31
Pandemic Prophylaxis.....	31
Pandemic Treatment.....	33
Global Management	33
Epidemic Management.....	33
Pandemic Management	34
Distribution	37
Conclusion	37
Golden Links	38
Interviews	38
References	38
Chapter 2: Avian Influenza.....	48
The Viruses.....	50
Natural hosts.....	53
Clinical Presentation.....	56
Pathology.....	57
LPAI.....	57
HPAI	58
Differential Diagnosis.....	58
Laboratory Diagnosis	59
Collection of Specimens.....	59

Transport of Specimens.....	59
Diagnostic Cascades.....	60
Transmission.....	62
Transmission between Birds	62
Poultry.....	66
Humans	67
Economic Consequences	67
Control Measures against HPAI	68
Vaccination.....	69
Pandemic Risk	71
Conclusion.....	72
References	73
Chapter 3: Virology of Human Influenza.....	87
Structure	87
Haemagglutinin.....	88
Neuraminidase.....	89
M2 protein.....	89
Possible function of NS1	90
Possible function of NS2.....	90
Replication cycle	90
Adsorption of the virus.....	90
Entry of the virus.....	90
Uncoating of the virus	90
Synthesis of viral RNA and viral proteins.....	91
Shedding of the virus and infectivity.....	91
References	91
Chapter 4: Pathogenesis and Immunology	92
Introduction	92
Pathogenesis	92
Viral entry: How does the virion enter the host?.....	93
Binding to the host cells.....	93
Where does the primary replication occur?	96
How does the infection spread in the host?	96
What is the initial host response?	97
How is influenza transmitted to others?	100
Immunology	100
The humoral immune response	102
The cellular immune response.....	103
Conclusion.....	105

References	105
Chapter 5: Pandemic Preparedness	110
Introduction	110
Previous Influenza Pandemics.....	110
H5N1 Pandemic Threat.....	110
Influenza Pandemic Preparedness	111
Pandemic Phases	111
Inter-Pandemic Period and Pandemic Alert Period	112
Surveillance.....	112
Implementation of Laboratory Diagnostic Services.....	113
Vaccines	114
Antiviral Drugs.....	115
General Measures.....	117
Pandemic Period.....	119
Surveillance.....	120
Treatment and Hospitalisation.....	120
Human Resources: Healthcare Personnel.....	120
Geographically Targeted Prophylaxis and Social Distancing Measures	120
Tracing of Symptomatic Cases.....	121
Border Control	121
Hygiene and Disinfection.....	122
Risk Communication.....	122
Conclusions	122
References	123
Chapter 6: Vaccines	127
Introduction	127
Vaccine Development.....	127
History.....	127
Yearly Vaccine Production	127
Types of Influenza Vaccine.....	129
Efficacy and Effectiveness	132
Side Effects.....	133
Recommendation for Use	133
Indications	133
Contraindications	135
Dosage / use	136
Companies and Products	137
Strategies for Use of a Limited Influenza Vaccine Supply.....	138
Antigen sparing methods.....	138

Rationing methods and controversies.....	139
Pandemic Vaccine	139
Development	139
Mock vaccines.....	140
Production capacity.....	141
Transition	141
Solutions.....	142
Controversies.....	143
Organising.....	143
The Ideal World – 2025	144
References	144
Useful reading and listening material.....	144
Sources	144
Chapter 7: Laboratory Findings	150
Introduction	150
Laboratory Diagnosis of Human Influenza.....	150
Appropriate specimen collection.....	150
Clinical role and value of laboratory diagnosis.....	150
Laboratory Tests	151
Direct methods	151
Isolation methods	153
Serology	153
Rapid tests	154
Differential diagnosis of flu-like illness	156
Diagnosis of suspected human infection with an avian influenza virus	156
Introduction.....	156
Specimen collection	156
Virological diagnostic modalities.....	156
Other laboratory findings	157
New developments and the future of influenza diagnostics.....	157
Conclusion.....	157
Useful Internet sources relating to Influenza Diagnosis	158
References	158
Chapter 8: Clinical Presentation	160
Uncomplicated Human Influenza	160
Complications of Human Influenza.....	161
Secondary Bacterial Pneumonia.....	162
Primary Viral Pneumonia.....	162
Mixed Viral and Bacterial Pneumonia	162

Exacerbation of Chronic Pulmonary Disease.....	162
Croup.....	163
Failure of Recovery.....	163
Myositis.....	163
Cardiac Complications.....	163
Toxic Shock Syndrome.....	163
Reye’s Syndrome.....	164
Complications in HIV-infected patients.....	164
Avian Influenza Virus Infections in Humans.....	164
Presentation.....	165
Clinical Course.....	165
References.....	166
Chapter 9: Treatment and Prophylaxis.....	170
Introduction.....	170
Antiviral Drugs.....	170
Neuraminidase Inhibitors.....	170
M2 Ion Channel Inhibitors.....	173
Treatment of “Classic” Human Influenza.....	174
Antiviral Treatment.....	175
Antiviral Prophylaxis.....	175
Special Situations.....	177
Treatment of Human H5N1 Influenza.....	178
Transmission Prophylaxis.....	179
Global Pandemic Prophylaxis.....	180
Conclusion.....	181
References.....	182
Chapter 10: Drug Profiles.....	188
Amantadine.....	188
Pharmacokinetics.....	189
Toxicity.....	189
Efficacy.....	190
Resistance.....	190
Drug Interactions.....	190
Recommendations for Use.....	190
Summary.....	192
References.....	193
Oseltamivir.....	194
Introduction.....	194
Structure.....	195

Pharmacokinetics	195
Toxicity	196
Efficacy	197
Resistance.....	199
Drug Interactions.....	200
Recommendations for Use	200
Summary	201
References	202
Rimantadine.....	207
Introduction	207
Structure	208
Pharmacokinetics	208
Toxicity	208
Efficacy	209
Resistance.....	210
Drug Interactions.....	210
Recommendations for Use	210
Summary	211
References	211
Zanamivir	213
Introduction	213
Structure	214
Pharmacokinetics	214
Toxicity	215
Efficacy	216
Resistance.....	217
Drug Interactions.....	218
Recommendations for Use	218
Summary	219
References	219

Chapter 1: Influenza 2006

Bernd Sebastian Kamps and Gustavo Reyes-Terán

Influenza pandemics resemble major natural disasters: we know there will be another one, but we ignore both time and magnitude. In most other aspects, they are different. Earthquakes in Tokyo or San Francisco last from seconds to a couple of minutes – pandemics spread around the world in successive waves over months or a couple of years. And quite different are the consequences: an influenza pandemic may be a thousand times more deadly than even the deadliest tsunami.

As unpredictable as influenza pandemics are, as unpredictable is the virus itself. We know nothing about the pathogenic potential of the next pandemic strain. The next pandemic may be relatively benign, as it was in 1968 and 1957, or truly malignant, as was the 1918 episode. We don't know if the next pandemic will be caused by the current *bête noire*, H5N1, or by another influenza strain. We ignore how the next pandemic will evolve over time, how rapidly it will spread around the world, and in how many waves. We don't know which age groups are at the highest risk of severe outcomes. We have no idea whether the next pandemic will kill 2, 20, or 200 million people.

Not surprisingly, healthcare professionals are becoming sensitised to the risk of a new pandemic. The ongoing outbreak of H5N1 influenza among birds with occasional transmission to human beings is of major concern because of intriguing parallels between the H5N1 virus and the 1918 influenza strain. Should H5N1 acquire the capability of easy human-to-human transmissibility, even the most conservative scenario anticipates up to several 100 million outpatient visits, more than 25 million hospital admissions and several million deaths globally ([WHO Checklist 2005](#)).

It is wise to imagine and plan for the worst when facing an unknown threat. As the threat is global, strategies must be global – a tricky task when our planet is divided into more than two hundred nations. Dealing with nations and their leaders is like dealing with children in a kindergarten. In this difficult context, the WHO is performing an astonishing job.

In the following paragraphs, we shall take a look at the various facets of the war on influenza: the global and individual impact of the disease, the virus itself, and the individual and global management of what may one day turn out to be one of the most challenging healthcare crises in medical history. The most important thing to remember when talking about pandemic influenza is that its severe form has little in common with seasonal influenza. Pandemic influenza is not business-as-usual influenza. Bear this in mind. You wouldn't call a tiger a cat.

Global Impact

Epidemics and Pandemics

Influenza is a serious respiratory illness which can be debilitating and cause complications that lead to hospitalisation and death, especially in the elderly. Every

year, the global burden of influenza **epidemics** is believed to be 3–5 million cases of severe illness and 300,000–500,000 deaths. The risk of serious illness and death is highest among persons aged > 65 years, children aged < 2 years, and persons who have medical conditions that place them at increased risk of developing complications from influenza (CDC 2005).

New epidemic influenza A strains arise every 1 to 2 years by the introduction of selected point mutations within two surface glycoproteins: haemagglutinin (HA) and neuraminidase (NA). The new variants are able to elude human host defences and there is therefore no lasting immunity against the virus, neither after natural infection nor after vaccination, as is the case with smallpox, yellow fever, polio, and measles. These permanent and usually small changes in the antigenicity of influenza A viruses are termed “antigenic drift” and are the basis for the regular occurrence of influenza epidemics (Figure 1). In addition, there is now evidence that multiple lineages of the same virus subtype can co-circulate, persist, and reassort in epidemiologically significant ways (Holmes 2005).

In contrast to epidemics, **pandemics** are rare events that occur every 10 to 50 years. They have been documented since the 16th century (WHO 2005b), and in the last 400 years, at least 31 pandemics have been recorded (Lazzari 2004). During the twentieth century, three influenza pandemics occurred (table 1). Their mortality impact ranged from devastating to moderate or mild (Simonsen 2004). The 1918 pandemic was caused by a H1N1 virus of apparently avian origin (Reid 1999), whereas the subsequent pandemic strains – H2N2 in 1957 and H3N2 in 1968 – were reassortant viruses containing genes from avian viruses: three in 1957 (haemagglutinin, neuraminidase, and the RNA polymerase PB1) and two (haemagglutinin and PB1) in 1968 (Kawaoka 1989). These major changes in the antigenicity of an influenza virus are called “antigenic shift” (Figure 2).

Table 1: Antigenic Shifts and Pandemics*

	Designation	Resulting Pandemic	Death Toll
1889	H3N2	Moderate	?
1918	H1N1 (“Spanish”)	Devastating	50–100 million
1957	H2N2 (“Asian”)	Moderate	1 million
1968	H3N2 (“Hong Kong”)	Mild	1 million
?			

* H = haemagglutinin; N = neuraminidase

Influenza pandemics circulate around the globe in successive waves, and there is no way to prevent the spread of a new pandemic influenza virus. The new viral strain will eventually reach everywhere, and will infect practically every human being within a period of a few years. Seasonal excess mortality rates due to pneumonia and influenza may remain elevated for many years, as was shown in the A(H3N2)-dominated seasons in the decade after 1968, in persons aged 45–64 years in the United States (Simonsen 2004).

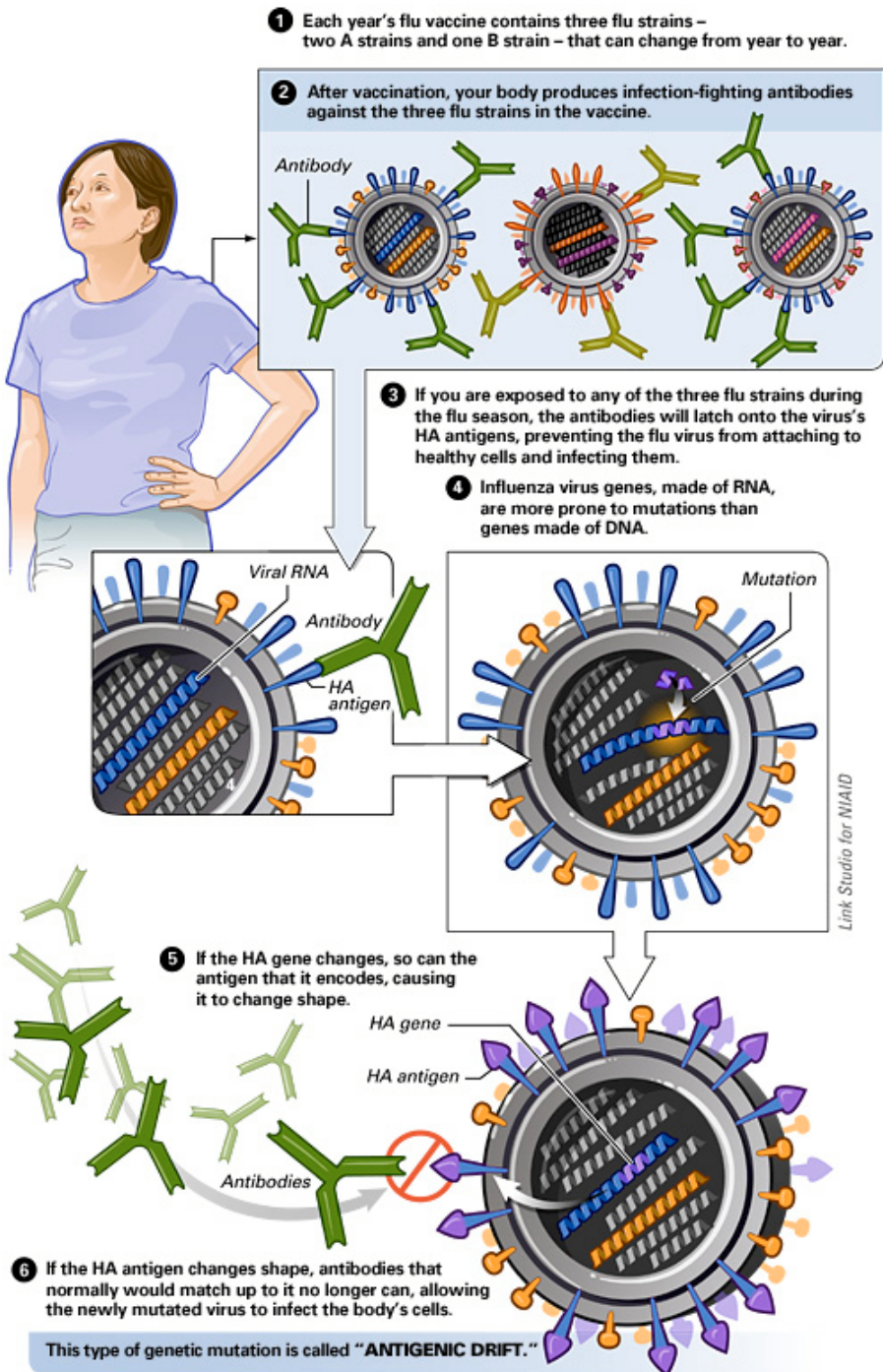


Figure 1. Antigenic drift. Courtesy: National Institute of Allergy and Infectious Disease

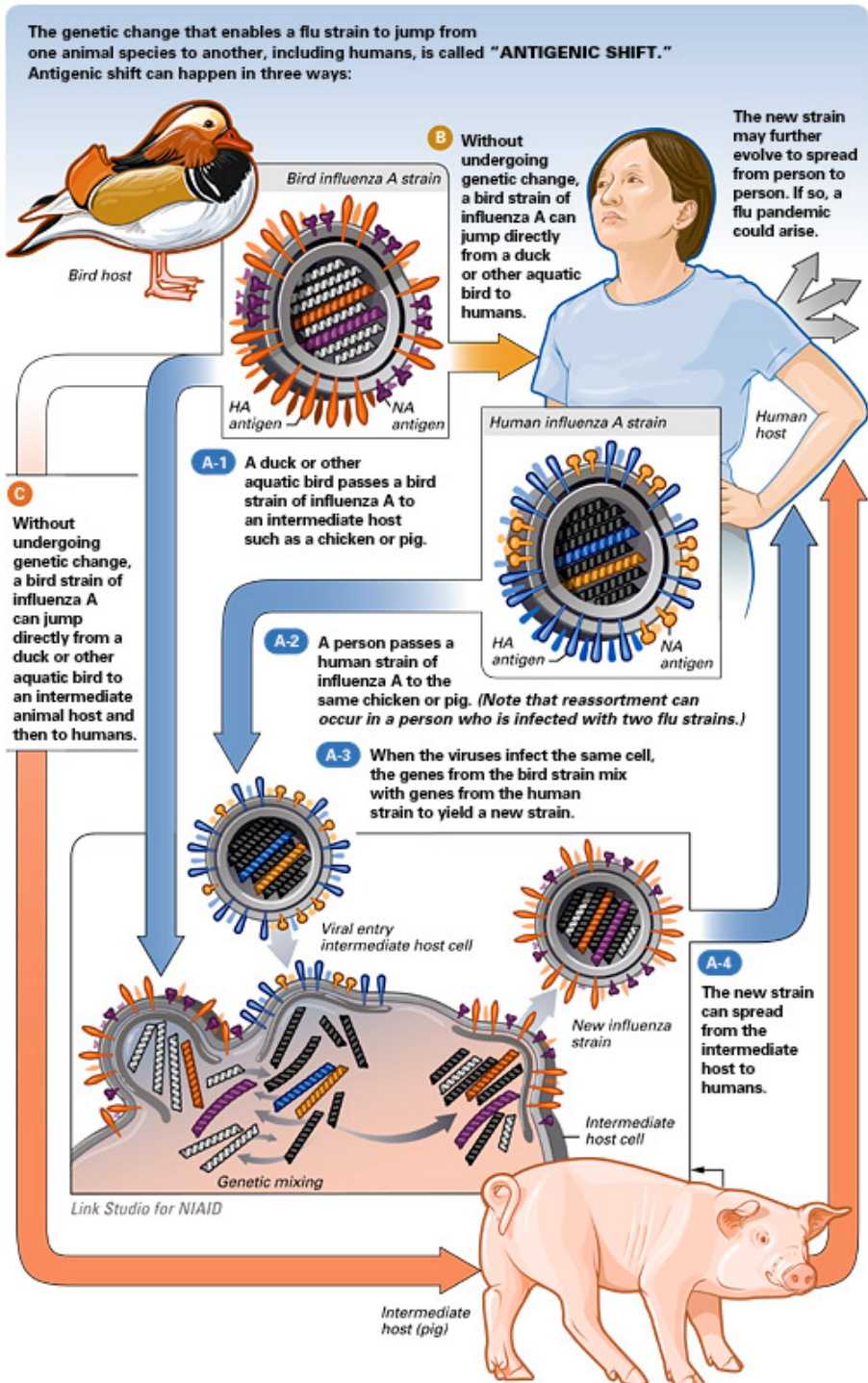


Figure 2. Antigenic shift. Courtesy: National Institute of Allergy and Infectious Disease

One hallmark of pandemic influenza is a mortality shift towards younger age groups. Half of influenza-related deaths during the 1968 pandemic, and large proportions of influenza-related deaths during the 1957 and the 1918 pandemics, occurred among persons < 65 years old (Simonson 1998).

1918

The first influenza pandemic of the 20th century spread more or less simultaneously in 3 distinct waves during a 12-month period in 1918–1919, across Europe, Asia, and North America (Barry 2004, Taubenberger 2006). It was the worst pandemic in history, killing more people than World War I, and it is generally assumed that at least 50 million people died (Johnson 2002). The first wave, which started during the spring of 1918, was highly contagious but not particularly deadly. Only the second wave, beginning in September, spread the deadly form of the pandemic.



Figure 3. Emergency hospital during influenza epidemic, Camp Funston, Kansas. Images from the 1918 Influenza Epidemic. Image copyright by National Museum of Health & Medicine, Washington, D.C. <http://InfluenzaReport.com/link.php?id=19>

The virus of 1918 was extremely virulent and caused many deaths through secondary bacterial pneumonia. The primary viral pneumonia could kill previously healthy young individuals within 2 days. The clinical course of severe cases was so unfamiliar that investigators doubted it was influenza (WHO 2005b). Symptoms in 1918 were so unusual that, initially, it was misdiagnosed as dengue fever, cholera, or typhoid (Barry 2004).

In less severe cases, most patients experienced typical influenza with a 3- to 5-day fever followed by complete recovery (Kilbourne 2006). In contrast to subsequent pandemics, most deaths during the 1918 pandemic were among young and healthy persons aged 15 to 35 years old, and 99 % of deaths occurred in people younger than 65 years.

The recovery of the genomic RNA of the 1918 virus from archived formalin-fixed lung autopsy material and from frozen, unfixed lung tissue from an influenza victim who was buried in permafrost in November 1918 (Taubenberger 1997) has enabled the complete coding of the sequences of all eight viral RNA segments of the 1918 H1N1 virus (Taubenberger 2005). According to this investigation, the 1918 virus was not a reassortant virus (like those of the 1957 and 1968 pandemics), but more likely an entirely avian-like virus that adapted to humans.

1957

The 1957 pandemic was caused by H2N2, a clinically milder virus than the one responsible for the 1918 pandemic. Outbreaks were frequently explosive, but the death toll was much lower. Mortality showed a more characteristic pattern, similar to that seen in seasonal epidemics, with most excess deaths confined to infants and the elderly (WHO 2005b). Patients with chronic underlying disease and pregnant women were particularly at risk of developing pulmonary complications (Louria 1957). The global excess mortality of the 1957 pandemic has been estimated at 1–2 million deaths.

1968

The 1968 pandemic, was also a mild pandemic. The mortality impact was not even particularly severe compared to the severe epidemic in 1967–1968 (the last H2N2 epidemic), as well as two severe H3N2 epidemics in 1975–1976, and in 1980–1981 (Simonsen 2004). The death toll has been estimated to have been around 1 million, and in the United States, nearly 50 percent of all influenza-related deaths occurred in the younger population under 65 years of age. Sero-archaeological studies showed that most individuals aged 77 years or older, had H3 antibodies before they were exposed to the new pandemic virus (Dowdle 1999) and that pre-existing anti-H3 antibodies might have protected the elderly (> 77 years old) during the 1968 H3N2 pandemic.

Since 1968, there has been only one episode – in 1976 – when the start of a new pandemic was falsely anticipated (Dowdle 1997, Gaydos 2006, Kilbourne 2006).

Current Situation

Major pandemics have occurred throughout history at an average of every 30 years and there is a general consensus that there will be another influenza pandemic. It is impossible to predict which influenza strain will be the next pandemic virus. One possible candidate is the avian H5N1 strain which has become endemic in wild waterfowl and in domestic poultry in many parts of Southeast Asia, and is recently spreading across Asia into Europe and Africa. Recent research has shown that just ten amino acid changes in the polymerase proteins differentiate the 1918 influenza virus sequences from that of avian viruses, and that a number of the same changes

have been found in recently circulating, highly pathogenic H5N1 viruses (Taubenberger 2005).

At present, H5N1 avian influenza remains largely a disease of birds. The species barrier is significant: despite the infection of tens of millions of poultry over large geographical areas for more than two years, fewer than 200 human cases have been confirmed by a laboratory (WHO 200601). Human cases, first documented in 1997 (Yuen 1998), coincided with outbreaks of highly pathogenic H5N1 avian influenza in poultry. Very limited human-to-human transmission of the H5N1 strain was documented in healthcare workers and family members with contact (Katz 1999, Buxton Bridges 2000). Although H5 antibodies were detected in these groups, indicating infection with the virus, no cases of severe disease occurred.

There are little data to show to what extent asymptomatic infection or mild clinical disease occur following infection with highly pathogenic avian H5N1 strains. If asymptomatic infections were frequent, the 55 % fatality rate of severe human H5N1 disease reported as of 21 March 2006 (WHO 20060321) would of course be less alarming. However, these episodes may be the exception, at least in some settings. In a study conducted in a Cambodian village with H5N1 outbreaks in poultry and 4 fatal human cases, testing of blood samples from 351 villagers found no additional infections, although many villagers had had significant exposure to infected poultry (ProMED 20060322.0893 and Buchy, personal communication).

Until now, the disease has predominantly affected children and young adults. Of 116 patients for whom demographical data had been published on the WHO Website from December 2003 until 9 February 2006, 50 % were 16 years old or younger, 75 % were younger than 30 years, and 90 % were younger than 40 years old (Promed 20060211.0463). The reason for this age distribution (exposure risk, disease reporting bias, intrinsic host issues, etc.) is unclear. Likewise, it is not known whether, and to what extent, genetic composition plays a role in the susceptibility and resistance to infection with H5N1 influenza virus (Promed 20060216.0512).

The next pandemic is expected to cause clinical disease in 2 billion people. Best-case scenarios, modelled on the mild pandemic of 1968, anticipate between 2 million and 7.4 million cases (WHO 2005b). However, if we translate the death toll associated with the 1918 influenza virus to the current population, there could be 180 million to 360 million deaths globally (Osterholm 2005).

Individual Impact

The fate of an individual during an influenza outbreak, be it epidemic or pandemic, is variable. It is estimated that about half of those infected have no clinical symptoms or signs. Among the others, clinical presentation varies from afebrile respiratory symptoms mimicking the common cold, to febrile illnesses ranging in severity from mild to debilitating (Hoffmann 2006a), and may cause disorders affecting the lung, heart, brain, liver, kidneys, and muscles (Nicholson 2003).

The clinical course is influenced by the patient's age, the degree of pre-existing immunity, properties of the virus, smoking, co-morbidities, immunosuppression, and pregnancy (Nicholson 2003). Death mostly occurs as a consequence of primary viral pneumonia or of secondary respiratory bacterial infections, especially in pa-

tients with underlying pulmonary or cardiopulmonary diseases. The very young and the elderly usually have the highest risk of developing serious complications; however, during pandemics, there is a mortality shift towards younger age groups (Simonson 1998).

In humans, replication of influenza subtypes seems to be limited to the respiratory epithelial cells. Once the virus enters a cell, it causes complex cytopathic effects, predominantly in the columnar epithelial cells, by shutting down the synthesis of host proteins. The loss of critical host cell proteins leads to cell death by necrosis (Yuen 2005). There are numerous individual factors associated with protection against or increasing the risk of a fatal outcome caused by a given influenza strain (Behrens and Stoll 2006), and genetic factors that affect host susceptibility are likely to play a role. Specific immunity against certain viral epitopes or some degree of cross-immunity may explain why people > 65 years were less affected by the 1918 pandemic. It is unknown whether similar mechanisms play a role in the curious age distribution of cases in the current outbreak of avian H5N1 influenza (ProMED 20060211.0463).

The unusual severity of H5N1 infection in humans was initially ascribed to multiple basic amino acids adjacent to the cleavage site, a feature characteristic of highly pathogenic avian influenza A viruses (Subbarao 1998). The presence of these basic amino acids renders the protein susceptible to proteases from many different types of tissues and allows extrapulmonary dissemination due to broadened tissue tropism (Yuen 2005). Another explanation may be that interferons are pivotal in preventing viral spread outside the respiratory tract and that H5N1 interferes with this innate defence against viral infection. It has been shown that the non-structural (NS) gene of highly pathogenic H5N1 viruses confers resistance to the antiviral effects of interferons and tumour necrosis factor alpha (Seo 2002). H5N1 viruses seem to induce higher gene transcription of pro-inflammatory cytokines than do H3N2 or H1N1 viruses, and are potent inducers of pro-inflammatory cytokines in macrophages, the most notable being TNF alpha (Cheung 2002). These mechanisms might ultimately lead to a cytokine storm and death (Peiris 2004).

In interpandemic influenza epidemics, recovery from interpandemic influenza is usually uneventful. In severe cases of human H5N1 influenza, however, mortality has so far been considerable (WHO 20060213). Dyspnoea, ARDS and multi-organ failure has been a dominant clinical feature in fatal cases (Hoffmann 2006a), with a median time from onset of symptoms to death of 9 days (n=76) (<http://www.influenzareport.com/links.php?id=16>).

The Virus

Infectious diseases are the result of a conflict of interest between macroorganisms and microorganisms. We are not alone on earth.

Requirements for Success

To become a pandemic strain, an influenza virus must comply with a series of requirements. It has to

- enter the human body and replicate there,
- cause illness in humans, and
- be easily transmittable between humans.

Ideally, it has to be more pathogenic than other competing influenza strains. In the current situation, the potential pandemic virus would have to compete with the already circulating H3N2 and H1N1 strains.

The prerequisite for success is good adaptation: adaptation to human cells; the capability to take over the production machinery of the host cell to produce new offspring; as well as making the individual cough and sneeze to spread the offspring viruses. The clue to success is virulence (Noah 2005, Obenauer 2006, Salomon 2006) – and novelty: if the virus is a true newcomer, most living human beings will have little or no protection at all. The new virus will have unlimited access to virtually every human being and will find a feeding ground of > 6.5 billion human beings. This is one of the biggest biomasses in the world.

The passing of powers from one reigning influenza subtype to a new one is called “antigenic shift” because the antigenic characteristics of the new virus need to shift dramatically to elude the immune system of virtually the entire mankind. Antigenic shift is a major change in the influenza A viruses resulting in new haemagglutinin and/or new neuraminidase proteins. This change may occur by: 1) reassortment of the segmented genome of two parent viruses, or 2) gradual mutation of an animal virus. For reassortment to take place, both the new pandemic candidate virus, normally of avian origin, and an already circulating human virus, i.e., H3N2 or H1N1, need to infect the same human host cell. Inside the cell, genes from both viruses are reassembled in an entirely new virus (they don’t actually have sex, but for didactic purposes, this image might work quite nicely). That’s what happened in 1957 and 1968 (Figure 2).

Reassortment may not be the best route for a candidate pandemic virus. Recent evidence with recombinant viruses containing genes from the 1918 pandemic virus shows that viruses expressing one or more 1918 virus genes were less virulent than the constellation of all eight genes together (Tumpey 2005). The 1918 virus was particular indeed: it appears that it was not the result of a reassortment of two existing viruses, but an entirely avian-like virus that gradually adapted to humans in stepwise mutations (Taubenberger 2005). It is obviously tempting to speculate that the emergence of a completely new human-adapted avian influenza virus in 1918 (n=1) could be deadlier than the introduction of reassortant viruses in 1957 and 1968 (n=2), but such speculation is not scientific. Interestingly – and worryingly –, some amino acid changes in the 1918 virus that distinguish it from standard avian sequences are also seen in the highly pathogenic avian influenza virus strains of H5N1, suggesting that these changes may facilitate virus replication in human cells and increase pathogenicity (Taubenberger 2005).

Virology

Influenza A and B viruses are enveloped viruses with a segmented genome made of eight single-stranded negative RNA segments of 890 to 2,341 nucleotides each (Gürtler 2006). They are spherical or filamentous in structure, ranging from 80 to 120 nm in diameter (Figure 4 and 5). When sliced transversely, influenza virions resemble a symmetrical pepperoni pizza, with a circular slice of pepperoni in the

middle and seven other slices evenly distributed around it (Noda 2006). On the basis of the antigenicity of the surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), influenza A viruses are further divided into sixteen H (H1–H16[Fouchier 2005]) and nine N (N1–N9) subtypes. HA is the major antigen for neutralising antibodies, and is involved in the binding of the virus to host cell receptors. NA is concerned with the release of progeny virions from the cell surface. Currently, only viruses of the H1N1 and H3N2 subtypes are circulating among humans.

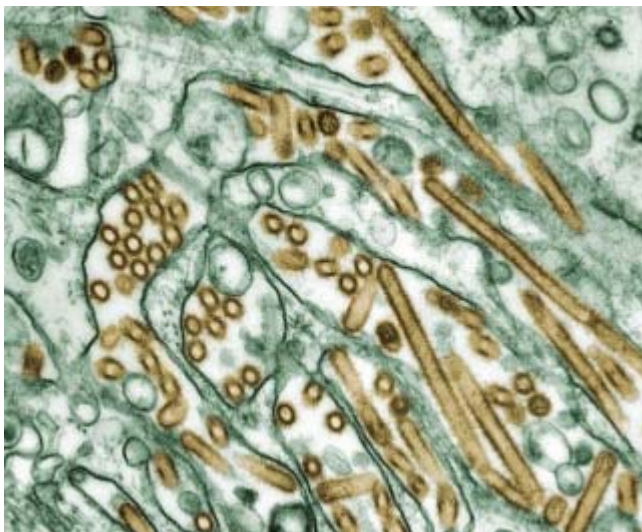


Figure 4. Colourised transmission electron micrograph of Avian influenza A H5N1 viruses (seen in gold) grown in MDCK cells (seen in green). Courtesy of CDC/ Cynthia Goldsmith, Jacqueline Katz, and Sharif R. Zaki, Public Health Image Library, <http://phil.cdc.gov/Phil/home.asp>

Natural Reservoir + Survival

Influenza A viruses occur in a large variety of species, mainly birds, notably aquatic ones, in which infection is largely intestinal, waterborne, and asymptomatic. The domestic duck in Southeast Asia is the principal host of influenza A viruses and also has a central role in the generation and maintenance of the H5N1 virus (Li 2004). In Thailand, there was a strong association between the H5N1 virus and the abundance of free-grazing ducks and, to a lesser extent, native chickens and cocks, as well as wetlands, and humans. Wetlands that are used for double-crop rice production, where free-grazing ducks feed year round in rice paddies, appear to be a critical factor in HPAI persistence and spread (Gilbert 2006).

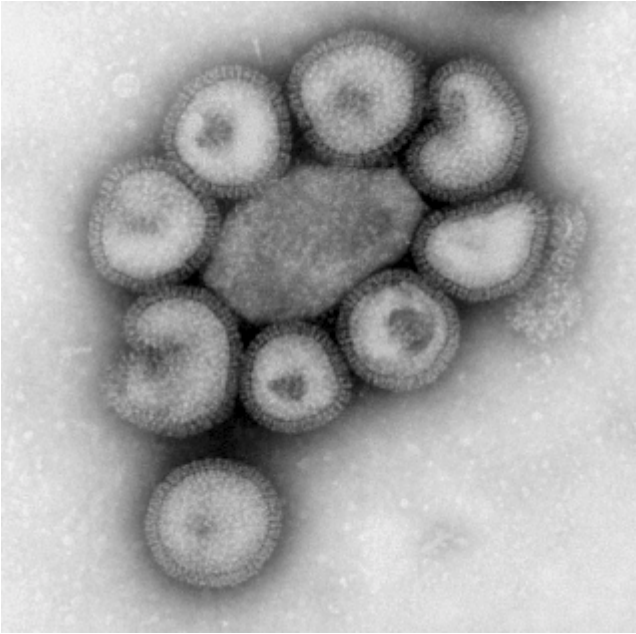


Figure 5. This negative-stained transmission electron micrograph (TEM) depicts the ultrastructural details of a number virions. Courtesy of CDC/ Dr. F. A. Murphy, Public Health Image Library, <http://phil.cdc.gov/Phil/home.asp>

Highly pathogenic avian viruses can survive in the environment for long periods, especially in low temperatures (i.e., in manure-contaminated water). In water, the virus can survive for up to four days at 22°C, and more than 30 days at 0°C. In frozen material, the virus probably survives indefinitely. Recent studies indicate that the H5N1 viruses isolated in 2004 have become more stable, surviving at 37°C for 6 days – isolates from the 1997 outbreak survived just 2 days (WHO 20041029). The virus is killed by heat (56°C for 3 hours or 60°C for 30 minutes) and common disinfectants, such as formalin and iodine compounds.

Transmission

Influenza is primarily transmitted from person to person via droplets (> 5 µm in diameter) from the nose and throat of an infected person who is coughing and sneezing (Figure 6). Particles do not remain suspended in the air, and close contact (up to 3–6 feet) is required for transmission. Transmission may also occur through direct skin-to-skin contact or indirect contact with respiratory secretions (touching contaminated surfaces then touching the eyes, nose or mouth). Individuals may spread influenza virus from up to two days before to approximately 5 days after onset of symptoms. Children can spread the virus for 10 days or longer.



Figure 6. An unimpeded sneeze sends two to five thousand bacteria-filled droplets into the air. Image copyright by Prof. Andrew Davidhazy, Rochester Institute of Technology. Used with permission. (<http://www.rit.edu/~andpph>)

As influenza viruses are normally highly species specific, they only rarely spill over to cause infection in other species. This is due to differences in the use of cellular receptors. Avian influenza viruses bind to cell-surface glycoproteins containing sialyl-galactosyl residues linked by a 2-3-linkage, whereas human viruses bind to receptors that contain terminal 2-6-linked sialyl-galactosyl moieties. For an avian virus to be easily transmitted between humans, it is fundamental that it acquires the ability to bind cells that display the 2-6 receptors so that it can enter the cell and replicate in them. While single amino acid substitutions can significantly alter receptor specificity of avian H5N1 viruses (Gambaryan 2006), it is presently unknown which specific mutations are needed to make the H5N1 virus easily and sustainably transmissible among humans, but potential routes whereby H5N1 might mutate and acquire human specificity do exist (Stevens 2006).

Since 1959, human infections with avian influenza viruses have only rarely occurred. Of the hundreds of strains of avian influenza A viruses, only four are known to have caused human infection: H5N1, H7N3, H7N7, and H9N2 (WHO 200601). Apart from H5N1, human infection generally resulted in mild symptoms and rarely in severe illness (Du Ry van Beest Holle 2003, Koopmans 2004). For the H5N1 virus, close contact with dead or sick birds (i.e., slaughtering, plucking, butchering and preparation) or exposure to chicken faeces on playgrounds seem to be the principal source of human infection (WHO 200601).

H5N1: Making Progress

At the moment, H5N1 infection in humans is relatively rare, although there must have been widespread exposure to the virus through infected poultry. This is an indicator that the species barrier to the acquisition of this avian virus is still quite high for H5N1 – despite having been in circulation for nearly 10 years. However, over the past years, H5N1 strains seem to have become more pathogenic and to have expanded their range of action:

- The H5N1 influenza strain continues to evolve (Li 2004), and some clones have broader binding properties which may reflect a certain degree of adaptation in human hosts (Le 2005). H5N1 has expanded its host range not only in avian species (Perkins 2002), but also in mammals, naturally infecting humans, tigers, leopards, domestic cats and a stone marten (Keawcharoen 2004, Thanawongnuwech 2005, Amonsin 2006).
- The H5N1 virus has increasingly pathogenic features in mice and ferrets (Zitzow 2002, Govorkova 2004).
- Ducks have recently been shown to be able to excrete highly pathogenic H5N1 strains for up to 17 days (Hulse Post 2005).
- In Central China, more than 6,000 migratory birds died at the Qinghai Lake nature reserve in central China in late April 2005. Before that event, it was highly unusual for wild birds to die from highly pathogenic avian influenza viruses (WHO 20050818).
- Viruses from very different locations (Qinghai Lake, Nigeria, Iraq, Turkey, Russia, Kazakhstan, and Mongolia) all showed a distinctive mutation which is associated with greater lethality in birds and mice. Such genetic stability over many months is unusual and raises the possibility that the virus – in its highly pathogenic form – has now adapted to at least some species of migratory waterfowl and is co-existing with these birds in evolutionary equilibrium, causing no apparent harm, and travelling with these birds along their migratory routes (WHO 20060220).
- In an unpublished study carried out in 2005 in central Thailand, 160 out of 629 dogs had antibodies to H5N1 (Butler 2006).
- Domestic cats are usually considered resistant to influenza. However, when fed with H5N1 virus-infected chickens, cats developed severe disease and transmitted the virus to other cats (Kuiken 2004). Cats may excrete virus not only via the respiratory tract but also via the digestive tract (Rimmelzwaan 2006), suggesting that spread by potentially novel routes within and between mammalian hosts might be possible. In February 2006, H5N1 influenza was found in a domestic cat (WHO 20060228) and in a stone marten (WHO 20060309) on the German island of Ruegen where more than 100 wild birds had died in the previous two weeks.
- Human H5N1 isolates from 2003 and 2004 exhibited a substantially greater level of virulence in ferrets than other H5N1 viruses isolated from humans since 1997 (Maines 2005).

Individual Management

Try not to get the bugs, and if you get them, try to treat them. In influenza management, this one-line medical wisdom theoretically translates as: 1) three prophylaxis defence lines (exposure prophylaxis, vaccination, prophylactic use of antiviral drugs); and 2) one treatment defence line (antiviral drugs). Due to the very nature of influenza infection – infected individuals may be infectious for as long as 24–48 hours before the onset of symptoms – exposure prophylaxis is virtually impossible during an ongoing epidemic or pandemic, especially in our highly

during an ongoing epidemic or pandemic, especially in our highly mobile and densely populated world.

Epidemic Prophylaxis

Exposure Prophylaxis

Basic personal hygiene measures, invented more than a century ago, are still the cornerstones of prophylaxis. Physicians should encourage regular hand-washing among family members of patients. In general, people should be discouraged to touch their eyes nose or mouth. Minimise the impact of sneezes and coughs by all possible means (WHO 2006a).

Vaccination

Vaccination against influenza viruses is the second cornerstone in preventing influenza. Vaccination in the northern hemisphere is recommended to start in October. Recommendations regarding the composition of the vaccine are issued yearly on the basis of detailed investigations of circulating strains. Vaccination against the prevalent wild-type influenza virus is recommended for all individuals in high-risk groups, including those aged 65 years or older (CDC 2005), and those with chronic illness, particularly diabetes, chronic respiratory and cardiac disease, and persons immunocompromised from disease or concomitant therapy. In addition, it is generally recommended that all healthcare personnel be vaccinated annually against influenza (CDC 2006b). The rate of influenza vaccination depends on a number of variables, including explicit physician recommendation and media coverage (Ma 2006).

In healthy primed adults, the efficacy after one dose may be as high as 80-100 %, while in unprimed adults (those receiving their first influenza immunisation), efficacy is in this range after two doses. With some underlying conditions (i.e., HIV infection, malignancies, renal transplantation), efficacy is lower (Korsman 2006); however, protection ultimately depends on who is vaccinated and on the match between the vaccine and the circulating virus (Wong 2005).

The evidence of efficacy and effectiveness of influenza vaccines in individuals aged 65 years or older has recently been reviewed. Well matched vaccines prevented hospital admission, pneumonia, respiratory diseases, cardiac disease, and death. The effectiveness is better in people living in homes for the elderly than in elderly individuals living in the community (Jefferson 2005). Inactivated vaccine reduces exacerbations in patients with chronic obstructive pulmonary disease (Poole 2006). Influenza vaccines are efficacious in children older than two years but little evidence is available for children under two (Smith 2006). Nasal spray of live vaccines seemed to be better at preventing influenza illness than inactivated vaccines.

Antiviral Drugs

In selected populations, antiviral drugs may be a useful option in those not covered or inadequately protected by vaccination. It should be emphasised, though, that the prophylactic use of available antiviral drugs is by no means a substitute for the yearly vaccination recommended by national health services.

Candidates for short-term prophylactic use of antiviral drugs are high-risk patients who are vaccinated only after an epidemic has already begun, as well as unvaccinated high-risk contacts of an individual with influenza. In some cases, prophylaxis could be indicated when a current epidemic is caused by a strain which is not represented in the vaccine. For more details, see [Hoffmann 2006b](#).

Of the two available drug classes, the adamantanes ([amantadine](#), [rimantadine](#)) recently came under pressure when the global prevalence of adamantane-resistant influenza viruses was found to have significantly increased from 0.4 % in 1994-1995 to 12.3 % in 2003-2004 ([Bright 2005](#)). It is believed that the elevated incidence of resistance in China is due to increased use of over-the-counter amantadine after the emergence of severe acute respiratory syndrome (SARS) ([Hayden 2006](#)). In the United States, 109/120 (91 %) influenza A (H3N2) viruses isolated in the 2005-06 season until January 12, 2006, contained an amino acid change at position 31 of the M2 protein, which confers resistance to amantadine and rimantadine ([CDC 2006](#), [Bright 2006](#)). On the basis of these results, the CDC issued an interim recommendation that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza A in the United States for the remainder of the 2005-06 influenza season. During this period, oseltamivir or zanamivir should be selected if an antiviral medication is used for the treatment and prophylaxis of influenza.

Epidemic Treatment

In uncomplicated cases, bed rest with adequate hydration is the treatment of choice for most adolescents and young adult patients ([Hoffmann 2006b](#)). Antibiotic treatment should be reserved for the treatment of secondary bacterial pneumonia.

The older drugs, [rimantadine](#) and [amantadine](#), are only effective against influenza A virus ([CDC 2005](#)). However, there is little data available on elderly people; the drugs have more side effects; and in the 2005/2006 season, the CDC **discouraged the use of these drugs** (see previous section). If rimantadine and amantadine are used, it is important to reduce the emergence of antiviral drug-resistant viruses. Amantadine or rimantadine treatment should therefore be discontinued as soon as possible, typically after 3-5 days of treatment, or within 24-48 hours after the disappearance of signs and symptoms ([CDC 2005](#)).

The newer neuraminidase inhibitors are licensed for treatment of patients aged 1 year and older ([oseltamivir](#)) or 7 years and older ([zanamivir](#)). They are indicated in patients with uncomplicated acute illness who have been symptomatic for no more than 2 days. The recommended duration of treatment for both drugs is 5 days.

Pandemic Prophylaxis

The problem with a new pandemic influenza strain is that there is no hiding place on earth. Virtually any single human being will eventually become infected with the new virus, be it the beggar from Paris or the President of a wealthy western country. If you don't get the virus during the first wave of the pandemic, you'll probably get it during the second. And if you don't get it during the second wave, you will get it during one of the future epidemics. If a novel pandemic influenza strain takes over as the driver of influenza disease in humans, everyone needs to mount a protective antibody response against the virus – simply because the virus is bound to stay with us for many years. Antibodies will provide some protection against the new influenza strain, but to develop antibodies you have to either be infected or vaccinated.

For the vast majority of the 6.5 billion living human beings, there will be no vaccine available any time soon after the arrival of a new pandemic influenza virus. Once a new virus has been shown to be effectively transmitted among humans, it will take approximately 6 months to start the production of the corresponding vaccine. Thereafter, vaccine supplies will be exquisitely inadequate, and years will be needed to produce enough vaccine for 6.5 billion people. In addition, production capacities are concentrated in Australia, Canada, France, Germany, Italy, Japan, the Netherlands, the United Kingdom, and the United States, and vaccine distribution can be expected to be controlled by the producing nations (Fedson 2005). We can all imagine who will be served first.

It is therefore reasonable to assume that the vast majority of people living today will have no access to either vaccine or antiviral drugs for many, many months. With no vaccine available or vaccine arriving too late, individuals might wish to work out strategies to deal with a pandemic situation. To confront or to avoid – that will be the question many people will ask themselves.

Simply confronting a new pandemic virus and hoping for a happy outcome, leaves the problem of timing. Indeed, there is conflicting evidence about the most adequate moment for getting infected:

- In the 1918 epidemic, the first wave which occurred during the spring months, was less deadly than the second, autumn wave (Barry 2004). It is reasonable to believe that people infected during the first wave had some protection during the second wave. That would speak in favour of confronting a new influenza strain as fast as possible.
- However, more detailed data from the second wave in 1918 suggest the contrary: the later someone got sick in the course of the second wave, the less likely he or she was to die, and the milder the illness tended to be (Barry 2004). Cities struck later generally suffered less, and individuals in a given city struck later also tended to suffer less. Thus, the West Coast American cities, hit later, had lower death rates than the East Coast cities; and Australia, which was not hit by the second wave until 1919, had the lowest death rate of any developed country (Barry 2004).

A commonly observed phenomenon in infectious diseases is that pathogens become less virulent as they evolve in a human population. This would favour the second option, i.e., of avoiding a new influenza virus for as long as possible. An additional advantage of this choice is that several months after the start of the pandemic, the initial chaos the health systems will inevitably face during a major outbreak, will have at least partially resolved.

The most extreme option of avoiding influenza would be to flee to remote areas of the globe – a mountain village in Corsica, the Libyan Desert, or American Samoa (Barry 2004). That might work but it might not. If the direct and unprotected confrontation with the new virus becomes inevitable, some protection is still possible: face masks (but: will masks be available everywhere? and for how long?) and social distancing (don't go to meetings, stay at home as much as possible) – but what if you are working as a cashier in a crowded Paris supermarket; as a metro driver in London's tube; as a clerk in Berlin's central post office?. Where will you get money from if you don't go to work for several months? Can you retire from the world? Can you retire from life?

Pandemic Treatment

We don't know whether the next pandemic influenza strain will be susceptible to the currently available antiviral drugs. If it is caused by a H5N1 virus, the neuraminidase inhibitors **oseltamivir** and **zanamivir** may be critical in the planning for a pandemic (Moscona 2005). Again, most people on earth will not have access to these drugs. They are in short supply and production capacities cannot easily be built up. Even in countries which have stockpiled oseltamivir, distribution of a drug that is in short supply will pose considerable ethical problems for treatment. In some countries with pronounced wealth disparities (i.e., some African and Latin American countries; the U.S.), social unrest can be anticipated.

Experience in treating H5N1 disease in humans is limited and the clinical reports published to date include only a few patients (Yuen 1998, Chan 2002, Hien 2004, Chotpitayasunondh 2005, WHO 2005, de Jong 2005). In particular, the optimal dose and duration of oseltamivir treatment is uncertain in H5N1 disease, and the following preliminary recommendations have been proposed (WHO 2005):

- Start treatment with oseltamivir as soon as possible. As H5N1 infections continue to have a high mortality rate, consider treatment even as late as 8 days after onset of symptoms, if there is evidence of ongoing viral replication (WHO 2005, de Jong 2005)
- Consider increasing the dose of oseltamivir in severe disease (150 mg twice daily in adults) and continue treatment for longer periods (7–10 days or longer) (WHO 2006d)

Although oseltamivir is generally well tolerated, gastrointestinal side effects in particular may increase with higher doses, particularly above 300 mg/day (WHO 2006d). For more details, check Hoffmann 2006b.

Global Management

The management of an influenza outbreak is well-defined for epidemics, and less well-defined for pandemics.

Epidemic Management

The cornerstone of medical intervention in **interpandemic years** is vaccination (see summary at CDC 2005). As influenza viruses mutate constantly, vaccine formulations need to be re-examined annually. Vaccine production is a well-established procedure: throughout the year, influenza surveillance centres in 82 countries around the world watch circulating strains of influenza and observe the trends. The WHO then determines the strains that are most likely to resemble the strains in circulation during the next year's winter seasons, and vaccine producers start vaccine production. The decision on the composition of the next "cocktail" is made each year in February for the following northern hemisphere winter (WHO 2006b) and in September for the following southern hemisphere winter (for more details, see Korsman 2006 and the figure at <http://influenzareport.com/link.php?id=15>). Predicting the evolutionary changes of the viral haemagglutinin is not easy and not always successful. In years when the anticipated strain does not match the real world strain, protection from influenza vaccine may be as low as 30 %.

Pandemic Management

– See also [Reyes-Terán 2006](#) and [WHO 2006c](#) –

Serious **influenza pandemics** are rare and unpredictable events. Managing unedited situations requires some appreciation of the magnitude of the problems that lie ahead. The impact on human health may be highly variable and is expressed in the number of

- infected individuals
- clinically ill individuals
- hospitalised patients
- deaths.

It is generally assumed that during the first year of the next pandemic 2 billion people will become infected with the new virus and that half of them will have symptoms. Less accurate are the estimates of the number of people that will require hospitalisation and the death toll. During the 1957 and 1968 pandemics, the excess mortality has been estimated at around one million deaths each. In contrast, 50 million individuals are thought to have died from the 1918 influenza pandemic. Excess mortality during the last influenza pandemics varied from 26 to 2,777 per 100,000 population (Table 2). Adjusted for today's world population, these figures would translate into 1.7 million to 180 million deaths.

Table 2: Death toll in 20th century pandemics and projections for the next pandemic *

	Population	Death Toll	per 100,000 people
1918	1.8 billion	50 million	2,777
1957	3.8 billion	1 million	26
1968	4.5 billion	1 million	27
Next	6.5 billion	1.7 million	26
Next	6.5 billion	180 million	2,777

According to data from <http://www.census.gov/ipc/www/world.html> + http://www.prb.org/Content/NavigationMenu/PRB/Educators/Human_Population/Population_Growth/Population_Growth.htm

In countries such as France, Spain and Germany, the yearly mortality from all causes is around 900 deaths per 100,000 population. A devastating pandemic might therefore, in the course of only a few months, cause three times as many deaths as would normally occur in an entire year. Indeed, social and economic disruption would occur in all countries to varying extents. In a world of extensive mass media coverage of catastrophic events, the resulting atmosphere would probably come close to war-time scenarios. In contrast, a mild pandemic similar to the 1968 episode would go nearly unnoticed and without considerable impact on national healthcare systems and on the global economy.

The concern that the world might be in for a revival of the 1918 scenario is based on the observation that the currently spreading H5N1 virus shares disturbing characteristics with the virus of the 1918 pandemic ([Taubenberger 2005](#)). However, if

H5N1 is to be the candidate virus for the next devastating influenza pandemic, why has it not yet acquired the ability to spread easily between humans? Over the past years, H5N1 has had both the time and opportunities to mutate into a pandemic strain. Why hasn't it? And if it hasn't in nearly 10 years, why should it do so in the future? It is true that of the 16 influenza H subtypes, only three (H1, H2 and H3) are known to have caused human pandemics (1918, 1957, 1968, and probably 1889 [Dowdle 2006]), and it has even been hypothesised that H5 viruses are inherently incapable of transmitting efficiently from human to human. Shall we one day discover that H5 viruses are not good for human pandemics, because not all possible subtypes can reassort to form functional human pandemic strains? We don't know.

Apart from stepwise mutations that transform an avian influenza virus into a human influenza virus, reassortment is the second way in which new pandemic viruses are generated. The two pandemics that were triggered by this phenomenon occurred in 1957 and in 1968. Both were relatively mild and fundamentally different from what happened in 1918. There is some preliminary experimental evidence that reassortants of the 1918 virus might be less virulent than the co-ordinated expression of all eight 1918 virus genes (Tumpey 2005). Does that mean that pandemics resulting from reassortment events of a human and an avian virus are milder than pandemics caused by a virus which slowly accumulates mutations in order to "migrate" from water fowl hosts to human hosts? We don't know.

The revival of the 1918 catastrophe might also never happen. But the 1918 influenza pandemic did occur, and good planning means being prepared for the worst. As it is impossible to predict whether the next pandemic will result in ~20 or ~2,000 deaths per 100,000 people, the international community should prepare for the 2,000 figure. The three defence lines are containment, drugs, and vaccines.

Containment

Containment and elimination of an emergent pandemic influenza strain at the point of origin has been estimated to be possible by a combination of antiviral prophylaxis and social distance measures (Ferguson 2005, Longini 2005). To this purpose, the WHO has recently started creating an international stockpile of 3 million courses of antiviral drugs to be dispatched to the area of an emerging influenza pandemic (WHO 20000824).

If the pandemic cannot be contained early on during an outbreak, rapid intervention might at least delay international spread and gain precious time. Key criteria for the success of this strategy have been developed (Ferguson 2005). However, the optimal strategy for the use of stockpiled antiviral drugs is not known, because stopping a nascent influenza pandemic at its source has never before been attempted.

Drugs

Once a pandemic is under way – and vaccines have not yet become available – national responses depend on the availability of antiviral drugs. As demand for the drug will exceed supply, stockpiling of antiviral drugs, either in the form of capsules or the bulk active pharmaceutical ingredient, has been considered a viable option by some governments.

The debate over which drugs should be stockpiled is not over. Until now, mainly oseltamivir has been used to constitute stockpiles of neuraminidase inhibitors. After the recent isolation of oseltamivir-resistant isolates in serious H5N1 infection, other

antiviral agents to which oseltamivir-resistant influenza viruses remain susceptible, should be included in treatment arsenals for influenza A (H5N1) virus infections (de Jong 2005) – in other words: zanamivir.

The value of adamantanes for stockpiling is less clear. H5N1 isolates obtained from patients in China in 2003 and in one lineage of avian and human H5N1 viruses in Thailand, Vietnam, and Cambodia were resistant to adamantanes (Hayden 2006). However, isolates tested from strains circulating recently in Indonesia, China, Mongolia, Russia, and Turkey appear to be sensitive to amantadine (Hayden 2005).

With regard to the economical impact, there is some evidence that even stockpiling of the costly neuraminidase inhibitors might be cost-beneficial for treatment of patients and, if backed by adequate stocks, for short-term postexposure prophylaxis of close contacts (Balicer 2005). When comparing strategies for stockpiling these drugs to treat and prevent influenza in Singapore, the treatment-only strategy had optimal economic benefits: stockpiles of antiviral agents for 40 % of the population would save an estimated 418 lives and \$414 million, at a cost of \$52.6 million per shelf-life cycle of the stockpile. Prophylaxis was economically beneficial in high-risk subpopulations, which account for 78 % of deaths, and in pandemics in which the death rate was > 0.6 %. Prophylaxis for pandemics with a 5 % case-fatality rate would save 50,000 lives and \$81 billion (Lee 2006).

Once a pandemic starts, countries without stockpiles of antiviral drugs will probably be unable to buy new stocks. In this context it has been suggested that governments provide compulsory licensing provisions, permitting generic manufacturers to start producing antivirals locally under domestic patent laws or to import them from generic producers at affordable prices (Lokuge 2006). In Europe, some governments are trying to build up stocks of the neuraminidase inhibitor oseltamivir for 25 % of the population. The number of treatment doses required to achieve this degree of “coverage” are based on the daily standard treatment course of 75 mg bid for 5 days. However, if doses twice as high, prescribed over a period twice as long (WHO 2005, WHO 2006d) should turn out to be required in a substantial number of patients, a stockpile planned for 25 % of a population might melt away more rapidly than expected.

For detailed information about drug treatment of influenza, see Hoffmann 2006b.

Vaccines

In an ideal world, we would have 6.5 billion vaccine doses the day after the pandemic starts; in addition, we would have 6.5 billion syringes to inject the vaccine; and finally, we would have an unlimited number of health personnel to administer the vaccine.

We don't live in an ideal world. At present, the world has a production capacity of about 300 million trivalent influenza vaccines per year, most of which is produced in nine countries (Fedson 2005). 300 million trivalent influenza doses translate into 900 million univalent doses, enough to vaccinate 450 million people with an initial vaccination and a booster dose – if the H5N1 vaccine is sufficiently immunogenic...

Influenza vaccines are currently prepared in fertilised chicken eggs, a process which was developed over 50 years ago (Osterholm 2005). New technologies may one day be able to develop vaccines more (Palese 2006). A dream vaccine would provide broad-spectrum protection against all influenza A subtypes (Neiryneck 1999, Fiers

2004, De Filette 2006), but these vaccines are experimental and years away from industrial production.

Distribution

When drug and vaccine supplies are limited, healthcare authorities have to decide who gains access to the drugs and vaccines. Who should receive short-supply vaccines and antivirals first: young people or the elderly (Simonsen 2004)? If the standard used to measure effectiveness of medical intervention was “numbers of deaths prevented,” then perhaps the elderly should be given priority - assuming they can produce an adequate antibody response to the pandemic vaccine. But if the concern is to minimise the years-of-life-lost, then the vaccine may be better used in young and middle-aged adults (Simonsen 2004).

The Australian Government has acknowledged that, in the event of a pandemic, its own stockpile of antivirals will be limited and reserved for those on a confidential rationing list (Lokuge 2006). Who are they? Physicians, fire fighters, police forces – or politicians and other VIPs? Experts urge that a framework for determining priority groups be developed prior to the start of a pandemic and that such a scheme should be agreed on beforehand and be flexible enough to adapt to the likely level of disaster at hand (Simonson 2004).

Conclusion

The good news from epidemiological research is that past pandemics gave warning signs. In the spring of 1918, a pandemic wave occurred 6 months before the second deadly autumn wave (Olson 2005). The Asian H2N2 influenza virus was characterised by early summer, 1957, but significant mortality in the United States did not occur until October – and in 1968, the pandemic wave of mortality in Europe peaked a full year after the pandemic strain first arrived (Simonson 2004).

Epidemiological studies of the 20th century pandemics offer some insight into what can be expected when the next influenza pandemic occurs (Simonson 2004):

- Mortality impact is difficult to predict, but a shift to younger ages is highly likely and people under 65 years of age will account for a high proportion of these deaths.
- Pandemic influenza is not always like a sudden storm, followed by a return to clear skies. Instead, mortality rates can remain elevated for several years – during which time an effective vaccine would be in high demand.
- In all three pandemics in the twentieth century, the majority of associated deaths occurred 6 months to a year after the pandemic virus first emerged, suggesting that intense and timely surveillance of both age-specific mortality and new influenza viruses could provide sufficient time for production and distribution of vaccines and antivirals to prevent much, if not most, of the mortality impact.

The next pandemic will come, but we do not know when. We do not know how severe it will be. Will it be mild like the last two pandemics of 1968 and 1957, when the new pandemic strain resulted from the reassortment of the pre-existing human strains and an avian influenza strain? Or will it be as catastrophic as the 1918 pandemic?

Only the future will tell. Let's be prepared!

Golden Links

Influenza. Special Issue of the Journal of Emerging Infectious Diseases, 2006.

<http://www.cdc.gov/ncidod/EID/index.htm>

Pandemic Influenza: Confronting a Re-emergent Threat. Special Issue of the Journal of Infectious Diseases, 1997. <http://www.journals.uchicago.edu/JID/journal/contents/v176nS1.html>

Interviews

Interview with Dr. Jeffrey Taubenberger. Spanish and avian flu pandemics. Nature Podcast, 6 October 2006 – <http://www.nature.com/nature/podcast/v437/n7060/nature-2005-10-06.mp3>

Interview with Dr. Frederick Hayden on antiviral resistance in influenza viruses. 23 February 2006 – <http://content.nejm.org/cgi/content/full/354/8/785/DC1>

Interview with Dr. Anne Moscona on the clinical implications of oseltamivir resistance. 22 December 2005 – <http://content.nejm.org/cgi/content/full/353/25/2633/DC1>

Interview with Dr. Michael Osterholm on preparing for an influenza pandemic. 5 May 2005 – <http://content.nejm.org/cgi/content/full/352/18/1839/DC1>

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Chapter 2: Avian Influenza

Ortrud Werner and Timm C. Harder

Highly pathogenic avian influenza, or, as it was termed originally, ‘fowl plague’, was initially recognised as an infectious disease of birds in chickens in Italy, 1878 (Perroncito 1878). Due to a former hot spot in the Italian upper Po valley it was also referred to as ‘Lombardian disease’. Although Centanni and Savonuzzi, in 1901, identified a filtrable agent responsible for causing the disease, it was not before 1955 that Schäfer characterised these agents as influenza A viruses (Schäfer 1955). In the natural reservoir hosts of avian influenza viruses, wild water birds, the infection generally runs an entirely asymptomatic course as influenza A virus biotypes of low pathogenicity co-exist in almost perfect balance with these hosts (Webster 1992, Alexander 2000).

When low pathogenic avian influenza virus (LPAIV) strains are transmitted from avian reservoir hosts to highly susceptible poultry species such as chickens and turkeys (i.e., a transspecies transmission step!), only mild symptoms are induced in general. However, in cases where the poultry species supports several cycles of infection, these strains may undergo a series of mutation events resulting in adaptation to their new hosts. Influenza A viruses of the subtypes H5 and H7 not only run through a host adaptation phase but may have the capability to saltatorily switch by insertional mutations into a highly pathogenic form (highly pathogenic avian influenza viruses, HPAIV) inducing overwhelming systemic and rapidly fatal disease. Such HPAI viruses may arise unpredictably *de novo* in poultry infected with LPAI progenitors of H5 and H7 subtypes.

HPAI in poultry is characterised by a sudden onset, severe illness of a short duration, and a mortality approaching virtually 100 % in vulnerable species. Due to excessive economical losses to the poultry industry, HPAI receives immense attention in the veterinary world and is globally treated as a disease immediately notifiable on suspicion to the authorities. Because of their potential to give rise to HPAIV, LPAI caused by subtypes H5 and H7 is also considered notifiable (OIE 2005). Before 1997, HPAI was fortunately a rare disease, with only 24 recorded primary outbreaks globally since the 1950s (Table 1).

Recently, however, avian influenza acquired world-wide attention when a highly pathogenic strain of the subtype H5N1, which probably arose before 1997 in Southern China, gained enzootic status in poultry throughout South East Asia and unexpectedly ‘traversed interclass barriers’ (Perkins and Swayne 2003) when transmitted from birds to mammals (cats, swine, humans). Although not an entirely unprecedented event (Koopmans 2004, Hayden and Croisier 2005), the substantial number of documented cases in humans, associated with severe disease and several fatalities raised serious concerns about a pandemic potential of the H5N1 strain (Klempner and Shapiro 2004; Webster 2006). There are several further lines of evidence – which will be discussed below – suggesting that the H5N1 virus has acquired increased pathogenic potency for several mammal species. Justifiably, this has caused world-wide public concern (Kaye and Pringle 2005).

Table 1: Previous outbreaks of highly pathogenic avian influenza worldwide¹

Year	Country/area	Domestic birds affected	Strain
1959	Scotland	2 flocks of chickens (reported)	A/chicken/Scotland/59 (H5N1)
1963	England	29,000 breeder turkeys	A/turkey/England/63 (H7N3)
1966	Ontario (Canada)	8,100 breeder turkeys	A/turkey/Ontario/7732/66 (H5N9)
1976	Victoria (Australia)	25,000 laying hens, 17,000 broilers, 16,000 ducks	A/chicken/Victoria/76 (H7N7)
1979	Germany	1 flock of 600,000 chickens, 80 geese	A/chicken/Germany/79 (H7N7)
1979	England	3 commercial farms of turkeys (total number of birds not reported)	A/turkey/England/199/79 (H7N7)
1983– 1985	Pennsylvania (USA)*	17 million birds in 452 flocks; most were chickens or turkeys, a few partridges and guinea fowls	A/chicken/Pennsylvania/1370/83 (H5N2)
1983	Ireland	800 meat turkeys died; 8,640 turkeys, 28,020 chickens, 270,000 ducks were depopulated	A/turkey/Ireland/1378/83 (H5N8)
1985	Victoria (Australia)	24,000 broiler breeders, 27,000 laying hens, 69,000 broilers, 118,418 chickens of unspecified type	A/chicken/Victoria/85 (H7N7)
1991	England	8,000 turkeys	A/turkey/England/50-92/91 (H5N1)
1992	Victoria (Australia)	12,700 broiler breeders, 5,700 ducks	A/chicken/Victoria/1/92 (H7N3)
1994	Queensland (Australia)	22,000 laying hens	A/chicken/Queensland/667-6/94 (H7N3)
1994– 1995	Mexico*	total number of birds not available, 360 commercial chicken flocks were depopulated	A/chicken/Puebla/8623-607/94 (H5N2)
1994	Pakistan*	3.2 million broilers and broiler breeder	A/chicken/Pakistan/447/95 (H7N3)
1997	Hong Kong (China)	1.4 million chickens and various lesser numbers of other domestic birds	A/chicken/Hong Kong/220/97 (H5N1)
1997	New South Wales (Australia)	128,000 broiler breeders, 33,000 broilers, 261 emus	A/chicken/New South Wales/1651/97 (H7N4)
1997	Italy	Approx. 6,000 chickens, turkeys, guinea fowls, ducks, quails, pigeons, geese, pheasants	A/chicken/Italy/330/97 (H5N2)
1999– 2000	Italy*	413 farms, approx. 14 million birds	A/turkey/Italy/99 (H7N1)
2002– 2005	SE Asia*	China, Hong Kong, Indonesia, Japan, Kambodscha, Laos, Malaysia, Korea, Thailand, Vietnam, approx. 150 million birds	A/chicken/East Asia/2003-2005 (H5N1)
2002	Chile		A/chicken/Chile/2002 (H7N3)

Table 1: Previous outbreaks of highly pathogenic avian influenza worldwide¹

Year	Country/area	Domestic birds affected	Strain
2003	Netherlands*	The Netherlands: 255 farms, 30 million birds; Belgium: 8 farms, 3 million birds; Germany: 1 farm, 80,000 broilers	A/chicken/Netherlands/2003 (H7N7)
2004	Canada (B.C.)*	53 flocks, 17 million chickens	A/chicken/Canada-BC/ 2004 (H7N3)
2004	United States (TX)	6,600 broilers	A/chicken/USA-TX/2004 (H5N2)
2004	South Africa	23,700 ratites, 5,000 chickens	A/ostrich/S.Africa/2004 (H5N2)

¹ Modified from Capua and Mutinelli, 2001

* Outbreaks with significant spread to numerous farms, resulting in great economic losses. Most other outbreaks were associated with only restricted or no spread from the index farms.

The Viruses

Influenza viruses are spherically or longitudinally shaped enveloped particles with an up to eight-fold segmented, single-stranded RNA genome of negative polarity. Influenza viruses hold generic status in the *Orthomyxoviridae* family and are classified into types A, B or C based on antigenic differences of their nucleo- and matrix proteins. Avian influenza viruses (AIV) belong to type A. Excellent reviews on the structure and replication strategy of influenza viruses have been published recently (e.g. Sidoronko and Reichl 2005).

The main antigenic determinants of influenza A and B viruses are the haemagglutinin (H or HA) and the neuraminidase (N or NA) transmembrane glycoproteins, capable of eliciting subtype-specific and immune responses which are fully protective within, but only partially protective across, different subtypes. On the basis of the antigenicity of these glycoproteins, influenza A viruses currently cluster into sixteen H (H1 – H16) and nine N (N1 – N9) subtypes. These clusters are substantiated when phylogenetically analysing the nucleotide and deduced amino acid sequences of the HA and NA genes, respectively (Fouchier 2005).

The conventional nomenclature for influenza virus isolates requires connotation of the influenza virus type, the host species (omitted in the case of human origin), the geographical site, serial number, and year of isolation. For influenza virus type A, the haemagglutinin and neuraminidase subtypes are added in brackets. One of the parental avian strains of the current outbreaks of H5N1 of Asian lineage was isolated from a goose in the Chinese province, Guangdong: accordingly, it is designated A/goose/Guangdong/1/96 (H5N1) (Xu 1999) while the isolate originating from the first-documented human case of Asian lineage H5N1 infection from Hong Kong (Claas 1998) is referred to as A/HK/156/97 (H5N1).

The haemagglutinin, a glycosylated and acylated protein consisting of 562 – 566 amino acids, is incorporated in the viral envelope. The globular head of its membrane-distal, knob-like external domain is associated with binding to cellular receptors composed of oligosaccharides which terminally carry derivatives of neuraminic acid (Watowich 1994). The exodomain of the second transmembrane glyco-

protein, the neuraminidase (NA), exerts sialolytic enzymatic activity and liberates virus progeny captured at the surface of infected cells during egress. This function prevents viral aggregation during egress, and possibly also facilitates the drifting of the virus through the mucus layers of the targeted epithelial tissues leading to viral attachment (Matrosovich 2004a). This renders the neuraminidase an interesting target of antiviral agents (Garman and Laver 2004). Mutually attuned and coordinated actions of the antagonistic glycoprotein species HA and NA of a viral strain are pivotal for effective attachment and release processes of the virions (Wagner 2002).

Attachment to cell surface proteins of influenza A virions is achieved through mature trimerised viral HA glycoproteins. Attachment is stratified by recognition of distinct terminal sialic acid species (N-acetyl- or N-glycolylneuraminic acid), the type of glycosidic linkage to penultimate galactose (α 2-3 or α 2-6) and the composition of further inner fragments of sialyloligosaccharides present at the cell surface (Herrler 1995, Gambaryan 2005). A variety of different sialyloligosaccharides are expressed with restriction to tissue and species origin in the different hosts of influenza viruses. Adaptation in both the viral HA and the NA glycoprotein to the specific receptor type(s) of a certain host species is a prerequisite for efficient replication (Ito 1999, Banks 2001, Matrosovich 1999+2001, Suzuki 2000, Gambaryan 2004). This implies a re-shaping of the receptor binding units of the HA protein following interspecies transmission (Gambaryan 2006). A mechanistic overview of the diverse receptor types is given in figure 1. Avian influenza viruses generally show the highest affinities for α 2-3 linked sialic acid as this is the dominating receptor type in epithelial tissues of endodermic origin (gut, lung) in those birds that are targeted by these viruses (Gambaryan 2005a, Kim 2005). Human-adapted influenza viruses, in contrast, primarily access 2-6 linked residues which predominate on non-ciliated epithelial cells of the human airway. These receptor predilections define part of a species barrier preventing hassle-free transmission of avian viruses to humans (Suzuki 2000, Suzuki 2005). Yet recently, it has been shown that there is a population of ciliated epithelial cells in the human trachea which also carry avian receptor-like glycoconjugates at lower densities (Matrosovitch 2004b), and also chicken cells carry human-type sialyl receptors at low concentrations (Kim 2005). This might explain why humans are not entirely refractory towards infection with certain avian strains (Beare and Webster 1991). In pigs, and also in quails, both receptor types are present at higher densities which renders these species putative mixing vessels for avian and human strains (Kida 1994, Ito 1998, Scholtissek 1998, Peiris 2001, Perez 2003, Wan and Perez 2005).

Once successfully attached to a suitable receptor, the virion is internalised into an endosomal compartment by clathrin-dependent and -independent mechanisms (Rust 2004). The virus escapes degradation in this compartment by fusing viral and endolysosomal membranes: mediated by proton transport through the viral matrix-2 (M2) tunnel protein at pH values in the endosome of around 5.0, a cascade of steric rearrangements in the matrix-1 (M1) proteins and the homotrimeric HA glycoprotein complex commence. As a result, a highly lipophilic, fusogenic domain of each HA monomere is exposed which inserts itself into the endolysosomal membrane, thereby initiating fusion of viral and lysosomal membranes (Haqae 2005, Wagner 2005).

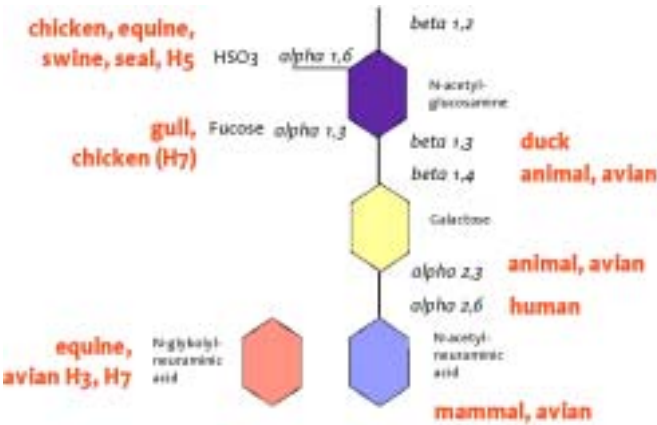


Figure 1. Overview of receptor predilections of influenza A viruses (based on data by Gambaryan 2005)

In turn, the eight viral genomic RNA segments, enclosed in a protective layer of nucleocapsid (N) proteins (ribonucleoprotein complex, RNP) are released into the cytoplasm. Here they are transported to the nucleus for transcription of viral mRNAs and replication of genomic RNA in a complex process which is delicately regulated by viral and cellular factors (Whittaker 1996). The RNA-dependent RNA polymerase (RdRp) is formed by a complex of the viral PB1, PB2 and PA proteins, and requires encapsidated RNA (RNPs) for this task. Upon translation of viral proteins and assembly of nucleocapsids harbouring replicated genomic RNA, progeny virions bud from the cellular membrane into which the viral glycoproteins have previously been inserted. Arrangements between helical nucleocapsids and viral envelope proteins are mediated by the viral matrix-1 (M1) protein which forms a shell-like structure just beneath the viral envelope. Viral reproduction in fully permissive cells is a fast (less than ten hours) and efficient process, provided an ‘optimal’ gene constellation is present (Rott 1979, Neumann 2004).

Due to the error-prone activity of the viral RdRp, a high mutation rate of $\geq 5 \times 10^{-5}$ nucleotide changes per nucleotide and replication cycle, thus approaching almost one nucleotide exchange per genome per replication, is observed among the influenza viruses (Drake 1993). In case selective pressures (such as neutralising antibodies, suboptimal receptor binding or chemical antivirals) are acting during viral replication on a host or population scale, mutants with corresponding selective advantages (e.g. escape from neutralisation, reshaped receptor-binding units) may be singled out and become the dominant variant within the viral quasispecies in that host or population. If antigenic determinants of the membrane glycoproteins HA and NA are affected by mechanisms driven by immunity, such a (gradual) process is referred to as *antigenic drift* (Fergusson 2003).

Antigenic shift, in contrast, denotes a sudden and profound change in antigenic determinants, i.e. a switch of H and/or N subtypes, within a single replication cycle. This occurs in a cell which is simultaneously infected by two or more influenza A viruses of different subtypes. Since the distribution of replicated viral genomic segments into budding virus progeny occurs independently from the subtype origin

of each segment, replication-competent progeny carrying genetic information of different parental viruses (so-called *reassortants*) may spring up (Webster and Hulse 2004, WHO 2005). While the pandemic human influenza viruses of 1957 (H2N2) and 1968 (H3N2) clearly arose through reassortment between human and avian viruses, the influenza virus causing the ‘Spanish flu’ in 1918 appears to be entirely derived from an avian source (Belshe 2005).

Natural hosts

Wild aquatic birds, notably members of the orders *Anseriformes* (ducks and geese) and *Charadriiformes* (gulls and shorebirds), are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses (Webster 1992, Fouchier 2003, Krauss 2004, Widjaja 2004). While all bird species are thought to be susceptible, some domestic poultry species – chickens, turkey, guinea fowl, quail and pheasants – are known to be especially vulnerable to the sequelae of infection.

Avian influenza A viruses generally do not cause disease in their natural hosts. Instead, the viruses remain in an evolutionary stasis, as molecularly signalled by low *N/S* (non-synonymous vs. synonymous) mutation ratios indicating purifying evolution (Gorman 1992, Taubenberger 2005). Host and virus seem to exist in a state of a meticulously balanced mutual tolerance, clinically demonstrated by absence of disease and efficient viral replication. Large quantities of virus of up to $10^{8.7}$ x 50% egg-infective dose (EID₅₀) per gram faeces can be excreted (Webster 1978). When transmitted to highly vulnerable poultry species, usually mild, if any, symptoms ensue. Viruses of this phenotype are referred to as low pathogenic (LPAIV) and, in general, only cause a slight and transient decline in egg production in layers or some reduction in weight gain in fattening poultry (Capua and Mutinelli 2001). However, strains of the subtypes H5 and H7 carry the potential to mutate to a highly pathogenic form *after* transmission and adaptation to the new poultry hosts. Nascency of highly pathogenic forms of H5 and H7 or of other subtypes has never been observed in wild birds (Webster 1998). Therefore, one may even come to look at the highly pathogenic forms as something artificial, made possible only as a result of man-made interference with a naturally balanced system.

Once HPAIV phenotypes have arisen in domestic poultry, they can be transmitted horizontally from poultry back into the wild bird population. The vulnerability of wild birds towards HPAIV-induced disease appears to vary grossly according to species, age and viral strain. Until the emergence of the Asian lineage H5N1 HPAI viruses, spill-overs of HPAIV into the wild bird population occurred sporadically and were locally restricted (with the single exception of a die-off among terns in South Africa in 1961 [Becker 1966]), so that wild birds had not been assigned an epidemiologically important function in the spread of HPAIV (Swayne and Suarez 2000). This might have changed fundamentally since early 2005, when a large outbreak of the Asian lineage H5N1-related HPAI was observed among thousands of wild aquatic birds in a nature reservation at Lake Qinghai in the North West of China (Chen 2005, Liu 2005). As a result of this, further spread of this virus towards Europe during 2005 may have been founded (OIE 2005). The details and consequences of this process are described below.

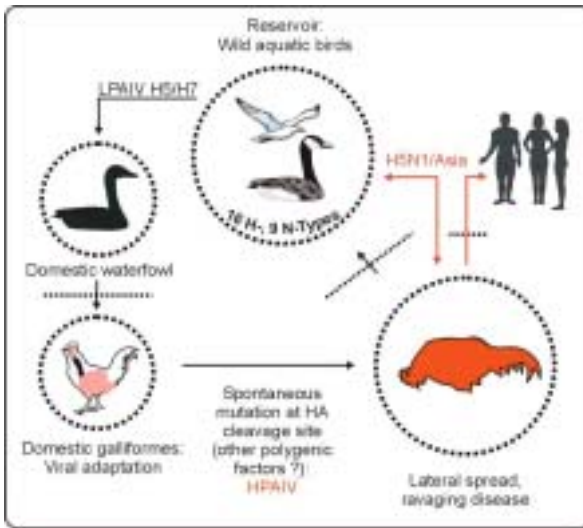


Figure 2. Scheme of avian influenza pathogenesis and epidemiology

LPAIV – low pathogenic avian influenza virus; HPAIV – highly pathogenic avian influenza virus; HA – haemagglutinin protein; dotted lines with arrows represent species barriers

Pathogenesis of HPAI

Pathogenicity as a general viral property in influenza A viruses is a polygenic trait and depends largely on an ‘optimal’ gene constellation affecting host and tissue tropism, replication efficacy and immune evasion mechanisms, amongst others. In addition, host- and species-specific factors contribute to the outcome of infection, which, after interspecies transmission, is therefore unpredictable *a priori*. The highly pathogenic form of avian influenza has been caused to date by influenza A viruses of the H5 and H7 subtypes exclusively. However, only a few representatives of the H5 and H7 subtypes in fact display a highly pathogenic biotype (Swayne and Suarez 2000). Usually, H5 and H7 viruses are stably maintained in their natural hosts in a low pathogenic form. From this reservoir, the viruses can be introduced by various pathways (see below) into poultry flocks. Following a variable and indecisive period of circulation (and, presumably, adaptation) in susceptible poultry populations, these viruses can saltatorily mutate into the highly pathogenic form (Rohm 1995).

Nucleotide sequencing studies have shown that most HPAIVs share a common feature in their HA genes which can serve, in poultry, as a virulence marker (Webster 1992, Senne 1996, Perdue 1997, Steinhauer 1999, Perdue and Suarez 2000):

In order to gain infectivity, influenza A virions must incorporate HA proteins which have been endoproteolytically processed from a HA₀ precursor to a disulphide-linked HA_{1,2} dimer (Chen 1998). The newly created N-terminus of the HA₂ subunit harbours a fusogenic peptide, composed of a highly lipophilic domain (Skehel

2001). This domain is vitally required during the fusion process of viral and lysosomal membranes because it initiates the penetration process of viral genomic segments into the host cell cytoplasm. The cleavage site of the HA of low pathogenic viruses is composed of two basic amino acids at positions -1/-4 (H5) and -1/-3 (H7) (Wood 1993). These sites are accessible to tissue-specific trypsin-like proteases which are preferentially expressed at the surface of respiratory and gastrointestinal epithelia. Therefore, efficient replication of LPAIVs is believed to be largely confined to these sites, at least in their natural hosts. In contrast, the cleavage site of HPAI viruses generally contains additional basic amino acids (arginine and/or lysine) which renders it processible for subtilysin-like endoproteases specific for a minimal consensus sequence of -R-X-K/R-R- (Horimoto 1994, Rott 1995). Proteases of this type (e.g. furin, proprotein-convertases) are active in virtually every tissue throughout the body. Therefore, viruses carrying these mutations have an advantage for replicating unrestrictedly in a systemic manner. This process has been documented in the field on several occasions. In Italy, for example, an LPAI H7N1 virus circulated for several months in the turkey and chicken population before, in December 1999, an HPAI H7N1 virus, distinguishable from its precursor only by its polybasic cleavage site, sprang up and caused devastating disease (Capua 2000).

It has been hypothesised that the HA gene of the H5 and H7 subtypes harbour distinct secondary RNA structures which favour insertional mutations (codon duplications) by a re-copying mechanism of the viral polymerase unit at a purine-rich sequence stretch encoding the endoproteolytic cleavage site of these HA proteins (Garcia 1996, Perdue 1997). This, and probably other mechanisms too, such as nucleotide substitutions or intersegmental recombination (Suarez 2004, Pasick 2005), may lead to the incorporation of additional basic amino acid residues. The latter has been experimentally proven by the generation of HPAIV from LPAIV precursors following repeated passaging *in vitro* and *in vivo* by site-directed mutagenesis (Li 1990, Walker and Kawaoka 1993, Horimoto and Kawaoka 1995, Ito 2001). Conversely, removal by reverse genetics of the polybasic cleavage site attenuates the HPAI phenotype (Tian 2005).

There are, however, viral strains in which the nucleotide sequence encoding the HA cleavage site and the pheno-/pathotype did not match in the predicted way: a Chilean H7N3 HPAIV which arose by intersegmental recombination displayed basic amino acid residues only at positions -1, -4 and -6 (Suarez 2004). Comparable examples exist for the H5 lineage (Kawaoka 1984). On the other hand, an H5N2 isolate from Texas was shown to harbour the HPAIV cleavage site consensus sequence, yet was clinically classified as LPAI (Lee 2005). These data re-emphasise the polygenic and intricate nature of influenza virus pathogenicity.

Fortunately, nascency of HPAI phenotypes in the field appears to be a rare event. During the last fifty years, only 24 primary HPAI outbreaks caused by HPAIV, which likely arose *de novo* in this way in the field, have been reported world-wide (Table 1).

In addition, HPAIV have been shown to be able to infect mammals, and humans in particular. This has especially been observed for the Asian lineage H5N1 (WHO 2006). Host-dependent pathogenicity of HPAIV H5N1 for mammals has been studied in several model species: mice (Lu 1999, Li 2005a), ferrets (Zitzow 2002, Govorkova 2005), cynomolgous monkeys (Rimmelzwaan 2001) and pigs (Choi 2005). The outcome of infection was dependent on the viral strain and species of

host. Ferrets appeared to mirror pathogenicity in humans better than mice (Maines 2005).

A number of genetic markers believed to be involved in pathogenicity have been located in different segments of the Z genotype of H5N1 (Table 2). Among these, mechanisms of interference with first-line defence mechanisms of the host, such as the interferon system, through the NS-1 gene product have received marked interest. Experimentally, it has been demonstrated using reverse genetics, that NS-1 proteins of some H5N1 strains carrying glutamic acid at position 92 are capable of circumventing the antiviral effects of interferon and tumour necrosis factor-alpha, eventually leading to enhanced replication in, and reduced clearance from, the infected host (Seo 2002+2004). In addition, immune-mediated damage resulting from NS-1-mediated disruption of cytokine networks may account for parts of the lung lesions (Cheung 2002, Lipatov 2005). However, none of the mutations (Table 2) on its own represents a true prerequisite for pathogenicity in mammals (Lipatov 2003). Therefore, optimal gene constellations, to a large extent, appear to drive pathotype specificities in a host-dependent manner in mammals (Lipatov 2004).

Table 2. Overview of genomic loci reported to be involved in enhanced mammalian pathogenicity of highly pathogenic Asian lineage H5N1 viruses

Gene, Protein	Mutation	Effects	Reference
HA	polybasic endo-proteolytic cleavage site	advantage for systemic dissemination and replication (poultry, mammals)	various
NA	19-25 aa deletion in stalk region	adaptation to growth in chickens and turkeys (?)	Matrosovich 1999, Giannecchini 2006
PB2	627K	enhanced systemic replication in mice	Hatta 2001, Shinya 2004
PB-1	701N	increased pathogenicity in mice	Li 2005
	13P, 678N		
NP	319K	enhanced polymerase activity; advantageous for early species-specific adaptation processes?	Gabriel 2005
NS-1	92E	facilitated escape of innate immune responses, reduced viral clearance in pigs	Seo 2004

Clinical Presentation

Following an incubation period of usually a few days (but rarely up to 21 days), depending upon the characteristics of the isolate, the dose of inoculum, the species, and age of the bird, the clinical presentation of avian influenza in birds is variable and symptoms are fairly unspecific (Elbers 2005). Therefore, a diagnosis solely based on the clinical presentation is impossible.

The symptoms following infection with low pathogenic AIV may be as discrete as ruffled feathers, transient reductions in egg production or weight loss combined

with a slight respiratory disease (Capua and Mutinelli 2001). Some LP strains such as certain Asian H9N2 lineages, adapted to efficient replication in poultry, may cause more prominent signs and also significant mortality (Bano 2003, Li 2005).

In its highly pathogenic form, the illness in chickens and turkeys is characterised by a sudden onset of severe symptoms and a mortality that can approach 100 % within 48 hours (Swayne and Suarez 2000). Spread within an affected flock depends on the form of rearing: in herds which are litter-reared and where direct contact and mixing of animals is possible, spread of the infection is faster than in caged holdings but would still require several days for complete contagion (Capua 2000). Often, only a section of a stable is affected. Many birds die without premonitory signs so that sometimes poisoning is suspected in the beginning (Nakatami 2005). It is worth noting, that a particular HPAI virus isolate may provoke severe disease in one avian species but not in another: in live poultry markets in Hong Kong prior to a complete depopulation in 1997, 20 % of the chickens but only 2.5 % of ducks and geese harboured H5N1 HPAIV while all other galliforme, passerine and psittacine species tested virus-negative and only the chickens actually showed clinical disease (Shortridge 1998).

In industrialised poultry holdings, a sharp rise followed by a progressive decline in water and food consumption can signal the presence of a systemic disease in a flock. In laying flocks, a cessation of egg production is apparent. Individual birds affected by HPAI often reveal little more than severe apathy and immobility (Kwon 2005). Oedema, visible at feather-free parts of the head, cyanosis of comb, wattles and legs, greenish diarrhoea and laboured breathing may be inconsistently present. In layers, soft-shelled eggs are seen initially, but any laying activities cease rapidly with progression of the disease (Elbers 2005). Nervous symptoms including tremor, unusual postures (torticollis), and problems with co-ordination (ataxia) dominate the picture in less vulnerable species such as ducks, geese, and ratites (Kwon 2005). During an outbreak of HPAI in Saxonia, Germany, in 1979, geese compulsively swimming in narrow circles on a pond were among the first conspicuous signs leading to a preliminary suspicion of HPAI.

The clinical presentation of avian influenza infection in humans is discussed in detail in the chapter entitled 'Clinical Presentation of Human Influenza'.

Pathology

LPAI

Lesions vary with the viral strain and the species and age of the host. In general, only turkeys and chickens reveal any gross and microscopic alterations especially with strains adapted to these hosts (Capua and Mutinelli 2001). In turkeys, sinusitis, tracheitis and airsacculitis have been detected, although secondary bacterial infections may have contributed as well. Pancreatitis in turkeys has been described. In chickens, mild involvement of the respiratory tract is most commonly seen. In addition, lesions concentrate on the reproductive organs of layers (ovaries, oviduct, yolk peritonitis).

HPAI

Gross pathological and histopathological alterations of HPAI reveal similar dependencies to those listed for the clinical presentation. Four classes of pathological alterations have been tentatively postulated (Perkins and Swayne 2003):

(i) Peracute (death within 24–36 hours post infection, mainly seen in some galliforme species) and acute forms of disease reveal no characteristic gross pathological alterations: a discrete hydropericardium, mild intestinal congestion and occasionally petechial bleedings of the mesenterical and pericardial serosa have been inconsistently described (Mutinelli 2003a, Jones and Swayne 2004). Chickens infected with the Asian lineage H5N1 sometimes reveal haemorrhagic patches and significant amounts of mucus in the trachea (Elbers 2004). Serous exudates in body cavities and pulmonary oedema may be seen as well. Pinpoint bleedings in the mucosa of the proventriculus, which were often described in text books in the past, have only exceptionally been encountered in poultry infected with the Asian lineage H5N1 (Elbers 2004). Various histological lesions together with the viral antigen can be detected throughout different organs (Mo 1997). The virus is first seen in endothelial cells. Later on virus-infected cells are detected in the myocardium, adrenal glands and pancreas. Neurons as well as the glial cells of the brain also become infected. Pathogenetically, a course similar to other endotheliotropic viruses may be assumed, where endothelial and leukocyte activation leads to a systemic and uncoordinated cytokine release predisposing to cardiopulmonary or multi-organ failure (Feldmann 2000, Klenk 2005).

(ii) In animals which show a protracted onset of symptoms and a prolonged course of disease, neurological symptoms and, histologically, non-suppurative brain lesions predominate the picture (Perkins and Swayne 2002a, Kwon 2005). However, virus can also be isolated from other organs. This course has been described in geese, ducks, emus and other species experimentally infected with an Asian lineage HPAI H5N1 strain. In laying birds, inflammation of the ovaries and oviducts, and, after follicle rupture, so-called yolk peritonitis, can be seen.

(iii) In ducks, gulls and house sparrows, only restricted viral replication was found. These birds showed mild interstitial pneumonia, airsacculitis and occasionally lymphocytic and histiocytic myocarditis (Perkins and Swayne 2002a, 2003).

(iv) In the experiments described by Perkins and Swayne (2003), pigeons and starlings proved to be resistant against H5N1 infection. However, Werner et al. (to be published) were able to induce protracted neurological disease, due to non-suppurative encephalitis (Klopfleisch 2006), in 5/16 pigeons using a recent Indonesian HPAI H5N1 isolate.

Differential Diagnosis

The following diseases must be considered in the differential diagnosis of HPAI because of their ability to cause a sudden onset of disease accompanied by high mortality or haemostasis in wattles and combs:

- a) velogenic Newcastle disease
- b) infectious laryngotracheitis (chickens)
- c) duck plague
- d) acute poisonings
- e) acute fowl cholera (Pasteurellosis) and other septicaemic diseases
- f) bacterial cellulitis of the comb and wattles

Less severe forms of HPAI can be clinically even more confusing. Rapid laboratory diagnostic aid, therefore, is pivotal to all further measures (Elbers 2005).

Laboratory Diagnosis

Collection of Specimens

Specimens should be collected from several fresh carcasses and from diseased birds of a flock. Ideally, adequate sampling is statistically backed up and diagnosis is made on a flock basis. When sampling birds suspected of HPAI, safety standards must be observed to avoid exposure of the sample collectors to potentially zoonanthropnotic HPAIV (Bridges 2002). Guidelines have been proposed by the CDC (CDC 2005).

For virological assays, swabs obtained from the cloaca and the oropharynx generally allow for a sound laboratory investigation. The material collected on the swabs should be mixed into 2-3 ml aliquots of a sterile isotonic transport medium containing antibiotic supplements and a protein source (e.g. 0.5 % [w/v] bovine serum albumin, up to ten percent of bovine serum or a brain-heart infusion).

At autopsy, carried out under safe conditions and avoiding spread of disease (see above), unpreserved specimens of brain, trachea/lung, spleen and intestinal contents are collected for isolation of the virus.

For serological purposes, native blood samples are taken. The number of samples collected should suffice detection with a 95 % confidence interval for a parameter with a prevalence of 30 %.

Transport of Specimens

Swabs, tissues and blood should be transported chilled but not be allowed to freeze. If delays of greater than 48 hours are expected in transit, these specimens should be frozen and transported on dry ice. In all cases, transport safety regulations (e.g. IATA rules) should be punctiliously observed to avoid spread of the disease and accidental exposure of personnel during transport. It is highly advisable to contact the assigned diagnostic laboratory before sending the samples and, ideally, even before collecting them.

Diagnostic Cascades

Direct Detection of AIV Infections

Basically, there are two (parallel) lines of diagnostic measures that attempt to (i) isolate and subtype the virus by classical methods (see [OIE Manual 2005](#)) and (ii) molecularly detect and characterise the viral genome.

(i) Conventionally, AI virus is isolated by inoculation of swab fluids or tissue homogenates into 9- to 11-day-old embryonated chicken eggs, usually by the chorio-allantoic sac route (Woolcock 2001). Depending on the pathotype, the embryos may or may not die within a five-day observation period and usually there are no characteristic lesions to be seen in either the embryo or the allantois membrane (Mutinelli 2003b). Eggs inoculated with HPAIV-containing material usually die within 48 hours. The presence of a haemagglutinating agent can be detected in harvested allantoic fluid. Haemagglutination (HA) is an insensitive technique requiring at least $10^{6.0}$ particles per ml. If only a low virus concentration is present in the inoculum, up to two further passages in embryonated eggs may be necessary for some LPAIV strains, in order to produce enough virus to be detected by HA. In the case of HPAIV, a second passage using diluted inoculum may be advantageous for the optimal production of haemagglutinating .

Haemagglutinating isolates are antigenically characterised by haemagglutination inhibition (HI) tests using (mono-) specific antisera against the 16 H subtypes and, for control, against the different types of avian paramyxoviruses which also display haemagglutinating activities. The NA subtype can be subsequently determined by neuraminidase inhibition assays, again requiring subtype-specific sera (Aymard 2003). In case isolates of the H5 or H7 lineages are encountered, their intravenous pathogenicity index (IVPI) needs to be determined to distinguish between LP and HP biotypes (Allan 1977). This is achieved by iv inoculation of ten 6-week old chickens with the egg-grown virus isolate (0.1 ml of a 1 in 10 dilution of allantoic fluid containing a HA titre greater than 1 in 16). The chickens are observed over a period of ten days for clinical symptoms. Results are integrated into an index which indicates a HPAI virus when values greater than 1.2 are obtained. Alternatively, a HPAI isolate is encountered when at least seven out of ten (75 %) inoculated chickens die within the observation period.

The described classical procedures can lead to a diagnosis of HPAI within five days but may demand more than a fortnight to rule out the presence of AIV. In addition, high quality diagnostic tools (SPF eggs, H- and N-subtype specific antisera) and skilled personnel are a prerequisite. Currently, there are no cell culture applications for the isolation of AIV that can achieve the sensitivity of embryonated hen eggs ([Seo 2001](#)).

(ii) A more rapid approach, especially when exclusion of infection is demanded, employs molecular techniques, which should also follow a cascade style: the presence of influenza A specific RNA is detected through the reverse transcription-polymerase chain reaction (RT-PCR) which targets fragments of the M gene, the most highly conserved genome segment of influenza viruses (Fouchier 2000, Spackman 2002), or the nucleocapsid gene (Dybkaer 2004). When a positive result is obtained, RT-PCRs amplifying fragments of the haemagglutinin gene of subtypes H5 and H7 are run to detect the presence of notifiable AIVs (Dybkaer 2004,

Spackman 2002). When positive again, a molecular diagnosis of the pathotype (LP versus HP) is feasible after sequencing a fragment of the HA gene spanning the endoproteolytic cleavage site. Isolates presenting with multiple basic amino acids are classified as HPAI. PCRs and other DNA detection techniques are being designed for the detection of Asian lineage H5N1 strains (Collins 2002, Payungporn 2004, Ng 2005). Non-H5/H7 subtypes can be identified by a canonical RT-PCR and subsequent sequence analysis of the HA-2 subunit (Phipps 2004). There are also specific primers for each NA subtype. A full characterisation might be achievable within three days, especially when real time PCR techniques are used (Perdue 2003, Lee and Suarez 2004). However, DNA chips are in development which should further streamline the typing of AI viruses (Li 2001, Kessler 2005). An exclusion diagnosis is possible within a single working day.

The disadvantages of molecular diagnostics are the price one has to pay for purchasing equipment and consumables, although, if available, many samples can be analysed by less personnel in grossly shorter times in comparison to virus isolation in eggs. However, it should not be kept secret that each PCR or hybridisation reaction, in contrast to virus isolation in eggs, harbours an intrinsic uncertainty related to the presence of specific mutations in a given isolate at the binding sites of primers and/or probes which might render the assay false negative.

Thus, a combination of molecular (e.g. for screening purposes) and classical methods (e.g. for final characterisation of isolates and confirmation of diagnosis of an index case) may help to counterbalance the disadvantages of the two principles.

Rapid assays have been designed for the detection of viral antigen in tissue impression smears and cryostat sections by use of immunofluorescence, or by antigen-capture enzyme-linked immunosorbent assay (ELISA) and dip-stick lateral flow systems in swab fluids. So far, these techniques have been less sensitive than either virus isolation or PCR, and therefore might be difficult to approve for a legally binding diagnosis, especially of an index case (Davison 1998, Selleck 2003, Cattoli 2004). The use of pen side tests in the veterinary field is still in its infancy and needs further development.

Indirect Detection of AIV Infections

Serology on a herd basis may be useful for screening purposes (Beck 2003). For the detection of AIV-specific antibodies in serum samples from birds, or in egg yolk in the case of laying flocks, the haemagglutination inhibition (HI) assay using reference subtype antigens still represents the gold standard. Group-specific antibodies (influenza virus type A) against the nucleocapsid protein can also be detected by agar gel immunoprecipitation and by enzyme-linked immunosorbent assays (ELISA) (Meulemans 1987, Snyder 1985, Jin 2004). Competitive ELISA formats allow the examination of sera of all bird species, independent from the availability of species-specific conjugates (Shafer 1998, Zhou 1998). An ELISA format for the detection of H7-specific antibodies has been reported (Sala 2003), but there is no such assay presently available for the detection of H5-specific antibodies in avian sera.

Subtype-specific antibody kinetics depend on the viral strain characteristics and, primarily, on the host species. In gallinaceous birds, AIV-specific antibodies reliably become detectable during the second week following exposure; antibodies in egg yolk are detectable after a delay of a few days (Beck 2003). The production and

detection of antibodies in *Anatidae* species are much more variable (Suarez and Shultz-Cherry 2000).

Transmission

Transmission between Birds

Avian influenza viruses of low pathogenicity circle genetically stable in wild water fowl (Webster 1992). The infection cycle among birds depends on faecal-oral transmission chains. Apart from being directly transmitted from host to host, indirect spread via virus-contaminated water and fomites is an important route in contrast to influenza virus infections in mammals (humans, swine, and horses) where transmission by aerosols prevails. In birds, peak excretion titres of up to $10^{8.7} \times 50\%$ egg-infective dose (EID₅₀) per gram faeces have been measured (Webster 1978). Average titres will be grossly lower. Avian influenza viruses reveal an astonishing capability to retain infectivity in the environment and particularly in surface water in spite of their seemingly delicate morphology (Stallknecht 1990a+b, Lu 2003). Virus suspensions in water have been shown to retain infectivity for more than 100 days at 17°C. Below -50°C the virus can be stored indefinitely. Data provided by Ito et al. (1995) and Okazaki et al. (2000) provided evidence that in the palearctic regions, avian influenza viruses are preserved in frozen lake water during the winter in the absence of their migrating natural hosts. Upon return for breeding purposes during the subsequent season, returning birds or their (susceptible) offspring are re-infected with viruses released by chance from melting environmental water. Along these lines, it has been hypothesised that influenza viruses can be preserved in environmental ice for prolonged time periods (Smith 2004), and that ancient viruses and genotypes might be recycled from this reservoir (Rogers 2004).

The introduction of H5 or H7 subtypes of LPAI viruses to susceptible poultry flocks is the basis of a chain of infection events which may lead to the *de novo* development of highly pathogenic biotypes. The risk that infection will be transmitted from wild birds to domestic poultry is greatest where domestic birds roam freely, share a water supply with wild birds, or use a water or food supply that might become contaminated by droppings from infected wild bird carriers (Capua 2003, Henzler 2003). Birds are infected by direct contact with virus-excreting animals and their excretions or through contact with (abiotic) vectors which are contaminated with virus-containing material. Once introduced into domestic flocks, LPAIV may or may not depend on a phase of adaptation to poultry species before they are excreted in amounts large enough to ensure sustained horizontal transmission within and between flocks. HPAIV, once it has arisen from an LPAIV infected flock, spreads by similar means. So-called 'wet' markets, where live birds are sold under crowded conditions, are multipliers of spread (Shortridge 1998, Bulaga 2003).

Biosecurity measures, aiming at the isolation of large poultry holdings, effectively prevent transmission from farm to farm by mechanical means, such as by contaminated equipment, vehicles, feed, cages, or clothing – especially shoes. An analysis of the Italian HPAI epizootic in 1999/2000 revealed the following risks for transmission: movements of infected flocks (1.0 %), mediated contacts during transport

of poultry to slaughter houses (8.5 %), neighbourhood within a one kilometre radius around infected premises (26.2 %), lorries used for transport of feed, bedding or carcasses (21.3 %), other indirect contacts through exchange of farm staff, working machines, etc. (9.4 %) (Marangon and Capua 2005). There were no hints at aerogenic spread obtained during the Italian epizootic. However, during outbreaks in the Netherlands (2003) and Canada (2004), airborne spread has been considered (Landman and Schrier 2004, Lees 2004). The role of live vectors such as rodents or flies, which may act as ‘mechanical vectors’ and are not themselves infected, is largely indetermined but certainly does not constitute a major factor.

Until the emergence of the Asian lineage H5N1 HPAIV, a re-introduction of HPAIV from poultry into the wild bird population had not played any significant role. In April 2005, however, Asian lineage H5N1-associated disease surfaced at Lake Qinghai in North Western China affecting thousands of bar-headed geese and other migratory species of ducks, cormorants and gulls (Chen 2005, Liu 2005). Therefore, transmission of Asian lineage H5N1 viruses by wild birds must be taken into account in future preventive concepts (discussed below).

Since late 2003, some H5N1 viruses have been encountered in Asia which were highly pathogenic for chickens but not for ducks (Sturm-Ramirez 2005). Experimental infections using these isolates revealed a heterogeneous mixture with respect to genetic analysis and plaque formation capacities in cell culture (Hulse Post 2005). Ducks that survived infection with these isolates were shown to shed a virus population on day 17 that had lost its pathogenic potential for ducks. When clinical signs are used to screen for the presence of HPAIV H5N1 in the field, ducks may become the ‘Trojan horse’ of this virus (Webster 2006).

Transmission to Humans

Transmission of avian influenza viruses to humans, leading to the development of clinically overt disease is a rare event (Table 3). Given the potential exposure of millions of people to HPAIV H5N1 in South East Asia, the actual number of documented human cases, although steadily growing over the past years, must still be considered as being comparatively low (http://www.who.int/csr/disease/avian_influenza/country/en).

The first association of the Asian lineage HPAIV H5N1 with respiratory illness in human beings was observed in Hong Kong in 1997, when six out of 18 H5N1 infected human cases died. These cases were epidemiologically linked to an outbreak of highly pathogenic H5N1 in live-bird markets (Yuen 1998, Claas 1998, Katz 1999). The risk of direct transmission of the H5N1 virus from birds to humans seems to be greatest in persons who have close contact with live infected poultry, or surfaces and objects heavily contaminated with their droppings. Exposure risk is considered substantial during slaughter, defeathering, butchering and preparation of poultry for cooking (http://www.who.int/csr/don/2005_08_18/en/). The Asian lineage HPAI H5N1 virus can be found in all tissues - including the meat - throughout the bird's carcass. In several such instances, it was reported that the person who slaughtered or prepared a sick bird for consumption developed fatal illness, while family members who participated in the meal did not (http://www.who.int/csr/don/2005_10_13/en/index.html).

Table 3. Documented human infections with avian influenza viruses*

Date	Country/Area	Strain	Cases (Deaths)	Symptoms	Source
1959	USA	H7N7**	1	respiratory	overseas travel
1995	UK	H7N7	1	conjunctivitis	pet ducks (shared lake with migratory birds)
1997	Hong Kong	H5N1**	18 (6)	respiratory/ pneumonia	poultry
1998	China (Guang-dong)	H9N2	5	unknown	unknown
1999	Hong Kong	H9N2	2	respiratory	poultry; unknown
2003 (Feb.)	Hong Kong	H5N1**	2 (1)	respiratory	unknown
2003 (Mar.)	Netherlands	H7N7**	89 (1)	conjunctivitis (pneumonia, respiratory insufficiency in fatal case)	poultry
2003 (Dec.)	Hong Kong	H9N2	1	respiratory	unknown
2003	New York	H7N2	1	respiratory	unknown
2003	Vietnam	H5N1**	3 (3)	respiratory	poultry
2004	Vietnam	H5N1**	29 (20)	respiratory	poultry
2004	Thailand	H5N1**	17 (12)	respiratory	poultry
2004	Canada	H7N3**	2	conjunctivitis	poultry
2005	Vietnam	H5N1**	61 (19)	respiratory	poultry
2005	Thailand	H5N1**	5 (2)	respiratory	poultry
2005	China	H5N1**	7 (3)	respiratory	poultry
2005	Cambodia	H5N1**	4 (4)	respiratory	poultry
2005	Indonesia	H5N1**	16 (11)	respiratory	poultry
2006	Turkey	H5N1**	3 (3)	respiratory	poultry

* Source: Avian influenza – assessing the pandemic threat. WHO, http://www.who.int/csr/disease/influenza/WHO_CDS_2005_29/en/, accessed 06 January 2006.

** Highly pathogenic for poultry

A H9N2 strain caused mild, influenza-like symptoms in two children in Hong Kong SAR in 1999, and in one child in mid-December 2003 (Saito 2001, Butt 2005). The H9N2 strain circulating in poultry at these times provoked significant symptoms and lethality rates in highly vulnerable species such as turkeys and chickens.

To date, there is no evidence that properly cooked poultry meat or poultry products are a source of human infection by the Asian lineage H5N1. As a general rule, the WHO recommends that meat be thoroughly cooked, so that all parts of the meat reach an internal temperature of 70°C. At this temperature, influenza viruses are inactivated, thus rendering safe any raw poultry meat contaminated with the H5N1 virus (WHO 2005).

Transmission to other Mammals

Avian influenza viruses have been transmitted to different mammal species on several occasions. Here, following cycles of replication and adaptation, new epidemic lineages can be founded. Pigs, in particular, have been frequently involved in such 'interclass transversions'. In European pig populations, avian-like H1N1 viruses are highly prevalent (Heinen 2002) and an H1N2 virus, a human-avian reassortant virus, first isolated in the U.K. in 1992, is constantly gaining ground (Brown 1998). In the U.S., a triple reassortant (H3N2) between the classical H1N1, the human H3N2 and avian subtypes is circulating (Olsen 2002). Other subtypes of presumably avian origin (e.g. H1N7, H4N6) have been found mainly anecdotally in swine (Brown 1997, Karasin 2000). A H9N2 virus of avian provenance is moderately prevalent in swine populations in the East of China (Xu 2004). In addition to swine, marine mammals and horses have been shown to acquire influenza A viruses from avian sources (Guo 1992, Ito 1999).

Natural infection with H5N1 was described in tigers and other large cats in a zoo in Thailand after the animals were fed with virus-positive chicken carcasses (Keawcharoen 2004, Quirk 2004, Amosin 2005). Severe disease accompanied by high mortality ensued. Also, cat-to-cat transmission has apparently occurred in the same zoo (Thanawongnuwech 2005). This was the first report of influenza virus infections in *Felidae*. Household European short hair cats can experimentally be infected with the H5N1 virus (Kuiken 2004).

In 2004, 3,000 serum samples obtained from free roaming pigs in Vietnam were tested serologically for evidence of exposure to the H5N1 influenza virus (Choi 2005). Virus neutralisation assay and Western blot analysis confirmed that only 0.25 % of the samples were seropositive. In experimental infections, it was shown that pigs can be infected with H5N1 viruses isolated in Asia in 2004 from human and avian sources. A mild cough and elevated body temperature were the only symptoms observed for four days post infection. Virus could be isolated from tissues of the upper respiratory tract for at least 6 days. Peak viral titres from nasal swabs were found on day 2 post infection, but none of the experimentally infected animals transmitted the infection to contact pigs. The highly lethal H5N1 viruses circulating in Asia seem to be capable of naturally infecting pigs. However, the incidence of such infections has been apparently low. None of the avian and human H5N1 viruses tested were readily transmitted between pigs under experimental conditions (Choi 2005). Based on these observations, pigs probably do not currently play an important role in the epidemiology of the Asian lineage H5N1.

An outbreak of the highly pathogenic H7N7 avian influenza in poultry, in the Netherlands, Belgium and Germany in Spring 2003, caused infection and mild illness, predominantly conjunctivitis, in 89 poultry workers exposed to infected animals and carcasses (Koopmans 2004). The infection of one veterinarian caused an acute respiratory distress syndrome and took a fatal course (Fouchier 2004). In addition, during the Dutch outbreak, H7N7 infection was virologically and serologically confirmed in several household contacts, four of which showed conjunctivitis (Du Ry van Beest Holle 2005). Evidence for (asymptomatic) natural infection with LPAIV strains of H9, H7 and H5 subtypes in humans has also been reported on other occasions in Italy and Japan (Zhou 1996, Puzelli 2005, Promed 20060110.0090).

In an anecdotal report (Promed Mail 20050826), a fatal infection due to H5N1 influenza in three rare civet cats born in captivity at a national park in Vietnam was mentioned. The source of the infection remained obscure. Another 20 civets of the same species, housed in adjacent cages, did not become sick.

Avian influenza viruses have never been detected in rats, rabbits and various other mammals present at live bird markets in Hong Kong where 20 % of the chickens were found positive for the Asian lineage H5N1 (Shortridge 1998).

Epidemiology

Poultry

Up to the end of 2003, HPAI was considered a rare disease in poultry. Since 1959, only 24 primary outbreaks had been reported world-wide (Table 1). The majority occurred in Europe and the Americas. Most outbreaks were geographically limited, with only five resulting in significant spread to numerous farms, and only one which spread internationally. None of the outbreaks had ever approached the size of the Asian outbreaks of H5N1 in 2004 (WHO 2004/03/02). To date, all outbreaks of the highly pathogenic form have been caused by influenza A viruses of the subtypes H5 and H7.

In the past outbreaks, illegal trade or movements of infected live birds or their unprocessed products, and unintended mechanical passing-on of virus through human movements (travellers, refugees, etc.) have been the main factors in the spread of HPAIV.

A new dimension of HPAI outbreaks became evident late in 2003. From mid-December 2003 through to early February 2004, outbreaks in poultry caused by the Asian lineage HPAI H5N1 virus were reported in the Republic of Korea, Vietnam, Japan, Thailand, Cambodia, Lao People's Democratic Republic, Indonesia, and China. The simultaneous occurrence in several countries of large epidemics of highly pathogenic H5N1 influenza in domestic poultry is unprecedented. All efforts aimed at the containment of the disease have failed so far. Despite the culling and the pre-emptive destruction of some 150 million birds, H5N1 is now considered endemic in many parts of Indonesia and Vietnam and in some parts of Cambodia, China, Thailand, and possibly also the Lao People's Democratic Republic.

The original virus, encountered for the first time in 1997, was of a reassortant parentage, including at least a H5N1 virus from domestic geese (A/goose/Guangdong/1/96, donating the HA) and a H6N1 virus, probably from teals (A/teal/Hong Kong/W312/97, donating the NA and the segments for the internal proteins), which underwent many more cycles of reassortation with other unknown avian influenza viruses (Xu 1999, Hoffmann 2000, Guan 2002b). Several different genotypes of the H5N1 lineage have been described (Cauthen 2000, Guan 2002a+2003). The so-called genotype 'Z' has dominated the outbreaks since December 2003 (Li 2004).

In April 2005, yet another level of the epizootic was reached, when, for the first time, the H5N1 strain obtained access to wild bird populations on a larger scale

(Chen 2005, Liu 2005). At Lake Qinghai in North Western China several thousands of bar-headed geese, a migratory species, succumbed to the infection. Several species of gulls as well as cormorants were affected as well at this location. When, in the summer and early autumn of 2005, H5N1 outbreaks were reported for the first time from geographically adjacent Mongolia, Kazakhstan, and Southern Siberia, migratory birds were suspected of spreading the virus. Further outbreaks along and between overlapping migratory flyways from inner Asia towards the Middle East and Africa hit Turkey, Romania, Croatia, and the Crimean peninsula in late 2005. In all instances (except those in Mongolia and Croatia) both poultry and wild aquatic birds were found to be affected. Often the index cases in poultry appeared to be in close proximity to lakes and marshes inhabited by wild aquatic birds. While this seems to suggest a direct hint towards migratory aquatic birds spreading the virus, it should be clearly noted that Asian lineage HPAI H5N1 virus has so far only been detected in moribund or dead wild aquatic birds. The true status of H5N1 in the populations of wild water birds and their role in the spread of the infection remains enigmatic. Presently, it can only be speculated as to whether wild aquatic birds can carry the virus over long distances during the incubation period, or whether some species indeed remain mobile despite an H5N1 infection.

Meanwhile, however, studies in China have revealed the presence of more new genotypes of the Asian lineage H5N1 virus in tree sparrows (Kou 2005). Neither the sparrows from which the viruses were isolated, nor the ducks that were experimentally infected with these viruses, showed any symptoms. However, upon transmission to chickens, full-blown HPAI was provoked. Since different sparrows of the same flock carried several distinguishable genotypes, which likely arose by reassortment with different AI viruses of unknown provenance, it was suspected that H5N1-like viruses had already been transmitted to these birds some time (months?) ago. These data mark another step of aggravation: sparrows, because of their living habits, are ideal mediators between wild birds and domestic poultry and may shuttle HPAI viruses between these populations. Locally restricted infection with HP H5N1 in individual (diseased or dead) sparrows has also been reported from Thailand and Kong Kong. Endemicity of HPAIV in passerine birds such as sparrows, starlings or swallows which live in close connection to human settlements would not only impose a huge pressure on local poultry industries but also increase the exposure risks for humans (Nestorowicz 1987).

Humans

Up until the 30th December 2005, 142 H5N1 cases in humans had been reported. The human epidemic is currently limited to Cambodia, Indonesia, Thailand, and the epicentre Vietnam (65.5 % of all cases). 72 (50.7 %) persons have died.

For more detailed information, see the chapter entitled “Epidemiology”.

Economic Consequences

Outbreaks of highly pathogenic avian influenza can be catastrophic for single farmers and for the poultry industry of an affected region as a whole (see Table 1). Economical losses are usually only partly due to direct deaths of poultry from HPAI infection. Measures put up to prevent further spread of the disease levy a heavy toll.

Nutritional consequences can be equally devastating in developing countries where poultry is an important source of animal protein. Once outbreaks have become widespread, control is difficult to achieve and may take several years (WHO 2004/01/22).

Control Measures against HPAI

Due to its potentially devastating economic impact, HPAI is subject world-wide to vigilant supervision and strict legislation (Pearson 2003, OIE *Terrestrial Animal Health Code* 2005). Measures to be taken against HPAI depend on the epidemiological situation of the region affected. In the European Union (EU) where HPAIV is not endemic, prophylactic vaccination against avian influenza is generally forbidden. Thus, outbreaks of HPAI in poultry are expected to be conspicuous due to the clinically devastating course of the disease. Consequently, when facing such an outbreak, aggressive control measures, e.g. stamping out affected and contact holdings, are put in place, aiming at the immediate eradication of HPAI viruses and containing the outbreak at the index holding.

For these purposes, control and surveillance zones are erected around the index case with diameters varying from nation to nation (3 and 10 kilometres, respectively, in the EU). The quarantining of infected and contact farms, rapid culling of all infected or exposed birds, and proper disposal of carcasses, are standard control measures to prevent lateral spread to other farms (OIE – *Terrestrial Animal Health Code*). It is pivotal that movements of live poultry and also, possibly, poultry products, both within and between countries, are restricted during outbreaks.

In addition, control of H5 and H7 subtypes of LPAI in poultry, by testing and culling of acutely infected holdings, may be advisable in non-endemic areas in order to reduce the risk of a de novo development of HPAIV from such holdings.

Specific problems of this eradication concept may arise in areas (i) with a high density of poultry populations (Marangon 2004, Stegemann 2004, Mannelli 2005) and (ii) where small backyard holdings of free roaming poultry prevail (Witt and Malone 2005). Due to the close proximity of poultry holdings and intertwining structures of the industry, spread of the disease is faster than the eradication measures. Therefore, during the Italian outbreak of 1999/2000 not only infected or contact holdings were destroyed, but also flocks with a risk of infection within a radius of one kilometre from the infected farm were pre-emptively killed. Nevertheless, eradication required four months and demanded the death of 13 millions birds (Capua 2003). The creation of buffer zones of one to several kilometres around infected farms completely devoid of any poultry was also behind the successful eradication of HPAIV in the Netherlands in 2003 and in Canada in 2004. So, not only the disease itself, but also the pre-emptive culling of animals led to losses of 30 and 19 million birds, respectively. In 1997, the Hong Kong authorities culled the entire poultry population within three days (on the 29th, 30th, and 31st December; 1.5 million birds). The application of such measures, aimed at the immediate eradication of HPAIV at the cost of culling also non-infected animals, may be feasible on commercial farms and in urban settings. However, this will afflict the poultry

industry significantly and also prompts ethical concern from the public against the culling of millions of healthy and uninfected animals in the buffer zones.

Such measures are most difficult to implement in rural areas with traditional forms of poultry holdings where chickens and ducks roam freely and mingle with wild birds or share water sources with them. Moreover, domestic ducks attract wild ducks and provide a significant link in the chain of transmission between wild birds and domestic flocks (WHO 2005). These circumstances may provide the grounds for HPAI viruses to gain an endemic status.

Endemicity of HPAI in a certain region imposes a constant pressure on poultry holdings. As the above mentioned restrictions can not be upheld over prolonged periods without vital damage to a country's poultry industry or, in the developing world, leading to a serious shortage of protein supply for the population, other measures must be considered.

Vaccination has been widely used in these circumstances and may also be a supplementary tool in the eradication process of outbreaks in non-endemic areas.

Vaccination

Vaccination in the veterinary world pursues four goals: (i) protection from clinical disease, (ii) protection from infection with virulent virus, (iii) protection from virus excretion, and (iv) serological differentiation of infected from vaccinated animals (so-called DIVA principle).

In the field of influenza vaccination, neither commercially available nor experimentally tested vaccines have been shown so far to fulfil all of these requirements (Lee and Suarez 2005). The first aim, which is the protection from clinical disease induced by HPAIV, is achieved by most vaccines. The risk of infection of vaccinees with, and excretion of, virulent field virus is usually reduced but not fully prevented. This may cause a significant epidemiological problem in endemic areas where exhaustive vaccination is carried out: vaccinated birds which appear healthy may well be infected and excrete the field virus 'under cover' of the vaccine. The effectiveness of reduction of virus excretion is important for the main goal of control measures, that is, the eradication of virulent field virus. The effectiveness can be quantified by the replication factor r_0 . Assuming a vaccinated and infected flock passes on the infection on average to less than one other flock ($r_0 < 1$), the virulent virus is, on mathematical grounds, prone to be extinguished (van der Goot 2005). When dealing with vaccination against the potentially zoonotic H5N1 virus, reduction of virus excretion also reduces the risks of transmission to humans, since a significant dose of virus seems to be required to penetrate the species barrier between birds and humans. Last but not least, a DIVA technique allows the tracing of field virus infections by serological means in vaccinated birds too.

For practical use several requirements must be observed (Lee and Suarez 2005):

- a) Due to their potency of genetic reassortment, as well as, in the case of H5- and H7-subtypes, a risk of spontaneous mutations leading to increased pathogenicity, vaccines are not to be composed of replication-competent influenza virus. Thus, live-attenuated vaccines are obsolete.
- b) Protection against HPAI in poultry largely depends on HA-specific antibodies. Therefore, the vaccine virus should belong to the same H subtype as the field virus. An ideal match of vaccine and field virus, as demanded for vaccine use in humans, is not mandatory in poultry. Induction of a homosubtypic cross-reactive immunity in poultry may be sufficient for protection, due to a current lack of vaccine-driven antigenic drift in avian influenza viruses, because of the absence of widespread vaccination.
- c) A marker (DIVA) strategy should be used (Suarez 2005). Alternatively, non-vaccinated sentinel birds may be used for monitoring.

A bunch of different vaccine concepts has been developed. Most are still based on inactivated, adjuvanted whole virus vaccines which need to be applied by needle and syringe to each animal separately.

Inactivated homologous vaccines, based on the actual HPAI strain, induce proper protection but do not allow a distinction of vaccinees and infected birds serologically. Since the vaccine is made from the current HPAI virus, there is an inherent delay before such vaccines can be used in the field.

Inactivated heterologous vaccines, in contrast, can be used as marker vaccines when the vaccine virus expresses the same HA- but a different NA-subtype compared to the field virus (e.g. H5N9 vaccine vs. H5N2 HPAI). By detection of NA subtype-specific antibodies, vaccinees and infected birds can be distinguished (Cattoli 2003). However, these methods can be laborious and may lack sensitivity. Nevertheless, such vaccines can be kept in vaccine banks comprising several H5- and H7-subtypes with discordant NA subtypes. Reverse genetics will greatly aid in producing vaccines both for veterinary and medical use with the desired HxNy combinations in a favourable genetic background (Liu 2003, Neumann 2003, Subbarao 2003, Lee 2004, Chen 2005, Stech 2005). Currently, inactivated heterologous vaccines are in field use in the H5N1 hot spots of South East Asia as well as in Mexico, Pakistan and Northern Italy (e.g. Garcia 1998, Swayne 2001). As an alternative DIVA system for use with inactivated vaccines, the detection of NS-1 specific antibodies has been proposed (Tumpey 2005). These antibodies are generated at high titres by naturally infected birds, but at considerably lower titres when inactivated vaccines are used.

Recombinant live vector-engineered vaccines express a H5 or H7 HA gene in the backbone of viruses or bacteria capable of infecting poultry species (e.g. fowl pox virus [Beard 1991, Swayne 1997+2000c], laryngotracheitis virus [Lueschow 2001, Veits 2003] or Newcastle Disease virus [Swayne 2003] among others). Being live vaccines, mass application via water or sprays is often feasible. While allowing for a clear-cut DIVA distinction, a pre-existing immunity towards the vector virus, however, will grossly interfere with vaccination success. Some field experience with fowl pox recombinants has been collected in Mexico and the U.S.

Finally, successful use of recombinantly expressed HA proteins and of DNA vaccination using HA-expressing plasmids has been experimentally proven (Crawford 1999, Kodihalli 1997).

Vaccination is now planned to be used on a nation wide scale in several countries in South East Asia (Normile 2005).

Pandemic Risk

Three conditions need to be met for a new pandemic to start:

- a) An influenza virus HA subtype, unseen in the human population for at least one generation, emerges (or re-emerges) *and*
- b) infects and replicates efficiently in humans *and*
- c) spreads easily and sustainably among humans.

This shows that a threat of a new human influenza pandemic is not uniquely linked to the emergence of HPAI H5N1. So far, H5N1 only meets two of these conditions: it is, for the vast majority of the human population, a new subtype and it has infected and caused severe illness and high lethality in more than 140 humans to date. There is no immunity against a H5N1-like virus in the vast majority of the human population. A new pandemic would be at the brink should the Asian lineage H5N1 acquire properties, by stepwise adaptation or by reassortment with an already human-adapted virus, for an efficient and sustained human-to-human transmission (Guan 2004). *In vitro*, it has been shown that two simultaneous amino acid exchanges in the receptor binding site of the HA protein of the Asian lineage HPAIV H5N1 (Q226L and G228S) optimises binding to human receptors of the 2-6 type like that of other human adapted influenza A viruses (Harvey 2004). Gambaryan et al. (2006) have already identified two human isolates originating from a father and his son infected with H5N1 in Hong Kong in 2003, which, in contrast to all other H5N1 isolates from humans and birds, showed a higher affinity for 2-6 receptors due to a unique S227N mutation at the HA1 receptor binding site.

This instance might be just around the corner or might already have occurred while reading this article – no one knows or can foretell. The chances for such an event to occur are directly correlated to the amount of virus circulating in poultry and, thus, the exposure risks of humans. Therefore, fighting H5N1 at its source would also reduce pandemic risks posed by this virus. Heretically, it has been proposed in one of the internet mail- and discussion-forums that the investment of only ten percent of the money that is scheduled to be spent for the development of H5-specific human vaccines in the eradication of H5N1 in poultry would have a greater effect than human vaccination in the protection of the human population from a H5N1 epidemic.

Since its first isolation in humans in 1997, H5N1 has failed to perform this last step towards pandemicity in human hosts. Recent studies, however, suggest that over the years, the virulence of H5N1 for mammals has increased and the host range has expanded:

1. H5N1 isolated from apparently healthy domestic ducks in mainland China from 1999 to 2002, and in Vietnam since 2003 have become progressively more pathogenic for mammals (Chen 2004).

2. H5N1 has expanded its host range, naturally infecting and killing mammalian species (cats, tigers) previously considered resistant to infection with avian influenza viruses (http://www.who.int/csr/don/2004_02_20/en/index.html).

However, it should not be overlooked that while staring at the H5N1 situation in Asia, other influenza viruses with possibly even greater pandemic potential may emerge or may already have emerged in the meantime. For example, strains of the H9N2 subtype which was not found in Asia prior to the 1980s have not only become widespread in Asian poultry populations, but also have crossed efficiently into pig populations in South Eastern and Eastern China (Shortridge 1992, Peiris 2001, Xu 2004). The receptor of these viruses revealed specificities similar to human-adapted viruses (Li 2005b, Matrosovich 2001). These H9 viruses have a broad host range, are genetically diverse and can directly infect man. The H9N2 strain, which was responsible for these human infections in Hong Kong, even revealed a genotype akin to that of the H5N1 viruses of 1997 (Lin 2000).

Conclusion

The importance of highly pathogenic avian influenza (AI) as a devastating disease of poultry has markedly increased during the last decade. The introduction of AI viruses of the subtypes H5 and H7 of low pathogenicity (LP) from a reservoir in wild water birds has been at the base of this process. It remains to be elucidated whether and, if so, why, the prevalence of LP H5 and H7 in their reservoirs has also been changing. With regard to the endemic status of the Asian lineage HPAI H5N1 in domestic poultry populations in South East Asia, causing frequent spill-overs into populations of migratory birds, a paradigm shift in the epidemiology of HPAI towards endemicity in migratory wild bird populations seems to be imminent. This would have grave consequences for the poultry industry on a transcontinental scale. Exposure risks for humans are directly linked to the increased presence of potentially zoonothronotic viruses in domestic poultry.

With respect to the avian and veterinary side of the story, many questions still remain unanswered:

1. Has the Asian lineage HPAIV H5N1 already established endemic status in populations of wild and migratory birds?
2. Can a HPAI virus evolve an attenuated phenotype in wild bird species whereby retaining its virulence for poultry?
3. Is there a role for land-based mammals in the spread of HPAIV?
4. Is the sequence stretch, encoding the endoproteolytical cleavage site of the HA protein, prone to mutations only in the subtypes H5 and H7?
5. What will be the impact of mass vaccination of poultry against H5N1 in Asia – prevention of viral spread or an acceleration of antigenic drift and escape?
6. Are shifts in the prevalence of LPAI subtypes H5 and H7 in their natural reservoirs potentially affecting also evolutionary stasis?

In particular, the first question is of overwhelming importance – not only for the veterinary world. Endemicity of the Asian lineage HPAIV H5N1 in migratory birds would pose a constant threat to poultry holdings. This would only be met by strict biosecurity measures including a prohibition of free-roaming poultry holdings. Alternatively, mass vaccination of poultry must be considered. As a second line, endemicity in wild birds may also lead to the presence of HPAI H5N1 virus in the environment (lakes, sea shores etc.) and might pose an additional potential risk of exposure for humans. So far, there are no reports of transmission from wild birds or environmental sources to humans. All reported human infections, including the most recent ones from Turkey, seemed to be acquired following virus amplification in, and close contact to, household poultry.

The complexity and the potential impact of the current, zoonothonotic HPAI H5N1 virus semi-pandemic in birds, demands concerted and prudent actions from scientists, politicians, and the public.

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74 Avian Influenza

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80 Avian Influenza

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82 Avian Influenza

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Chapter 3: Virology of Human Influenza

Lutz Gürtler

Human influenza viruses are members of the orthomyxovirus family, which consists of the genera: influenza A, B, and C virus, and Thogovirus (in ticks). In humans, only influenza A and B viruses are of epidemiological interest.

The main antigenic determinants of influenza A and B viruses are the haemagglutinin (H or HA) and neuraminidase (N or NA) transmembrane glycoproteins. Based on the antigenicity of these glycoproteins, influenza A viruses are further subdivided into sixteen H (H1–H16) and nine N (N1–N9) subtypes. The full nomenclature for influenza virus isolates requires connotation of the influenza virus type (A or B), the host species (omitted if human in origin), the geographical site, serial number, year of isolation, and lastly, the H and N variants in brackets, for example: A/goose/Guangdong/1/96 (H5N1).

Influenza viruses are usually transmitted via air droplets, and subsequently contaminate the mucosa of the respiratory tract. They are able to penetrate the mucin layer of the outer surface of the respiratory tract, entering respiratory epithelial cells, as well as other cell types. Replication is very quick: after only 6 hours the first influenza viruses are shed from infected cells. Part of the viral proteins, such as the fusion peptide and NS2, act as toxins to promote the production of influenza virus. Rapid bacterial growth, most commonly *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*, may begin in the very early phase of viral replication (for more details, see the chapter on [Pathogenesis](#)).

Structure

Influenza viruses are enveloped single-stranded RNA viruses with a pleomorphic appearance, and an average diameter of 120 nm. Projections of haemagglutinin and neuraminidase cover the surface of the particle (Figure 1).

The influenza A and B virus genomes consist of 8 separate segments covered by the nucleocapsid protein. Together these build the ribonucleoprotein (RNP), and each segment codes for a functionally important protein:

1. Polymerase B2 protein (PB2)
2. Polymerase B1 protein (PB1)
3. Polymerase A protein (PA)
4. Haemagglutinin (HA or H)
5. Nucleocapsid protein (NP)
6. Neuraminidase (NA or N)
7. Matrix protein (M): M1 constructs the matrix; and in influenza A viruses only, M2 acts as an ion channel pump to lower or maintain the pH of the endosome
8. Non-structural protein (NS); the function of NS2 is hypothetical

The active RNA-RNA polymerase, which is responsible for replication and transcription, is formed from PB2, PB1 and PA. It has an endonuclease activity and is

linked to the RNP. The NS1 and NS2 proteins have a regulatory function to promote the synthesis of viral components in the infected cell (see below).

The envelope of the virus is a lipid bilayer membrane which originates from the virus-producing cell and which contains prominent projections formed by HA and NA, as well as the M2 protein. The lipid layer covers the matrix formed by the M1 protein.

Influenza C virus harbours only 7 genome segments, and its surface carries only one glycoprotein. As it has a low pathogenicity in humans, it will not be discussed here in detail.

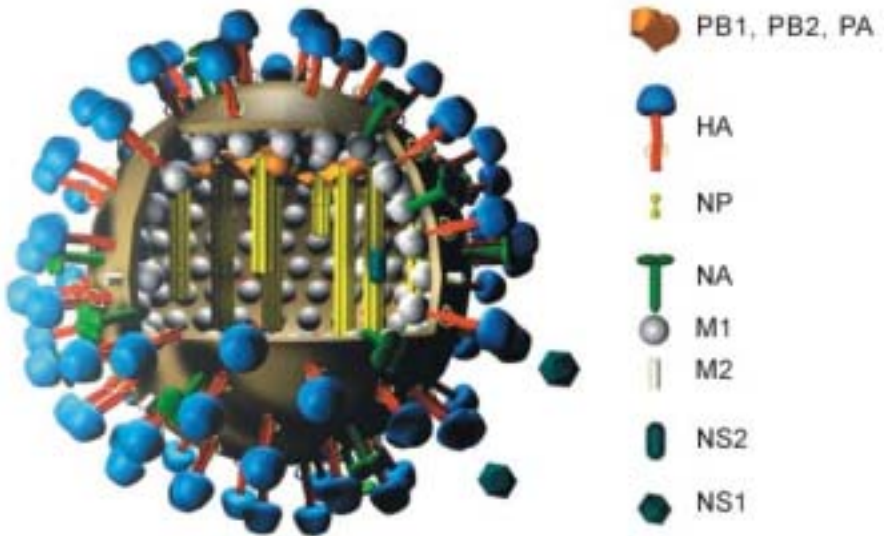


Figure 1. Structure of an influenza A virus. Image copyright by Dr. Markus Eickmann, Institute for Virology, Marburg, Germany. Used with permission. – <http://www.biografix.de>

Haemagglutinin

Haemagglutinin (HA or H) is a glycoprotein containing either 2 of 3 glycosylation sites, with a molecular weight of approximately 76,000. It spans the lipid membrane so that the major part, which contains at least 5 antigenic domains, is presented at the outer surface. HA serves as a receptor by binding to sialic acid (N-acetylneuraminic acid) and induces penetration of the interior of the virus particle by membrane fusion. Haemagglutinin is the main influenza virus antigen; the antigenic sites being A, B (carrying the receptor binding site), C, D, and E. The antigenic sites are presented at the head of the molecule, while the feet are embedded in the lipid layer. The body of the HA molecule contains the stalk region and the fusogenic domain which is needed for membrane fusion when the virus infects a new cell. At low pH, the fusion peptide is turned to an interior position. The HA forms trimers and several trimers form a fusion pore.

Prominent mutations in the antigenic sites reduce or inhibit the binding of neutralising antibodies, thereby allowing a new subtype to spread within a non-immune

population. This phenomenon is called **antigenic drift**. The mutations that cause the antigenic drift are the molecular explanation for the seasonal influenza epidemics during winter time in temperate climatic zones. The immune response to the HA antigenic sites is followed by the production of neutralising antibody, which is the basis for resolving infection in an individual, and is sometimes part of the cross immunity found in elderly individuals when a new pandemic virus strain occurs.

Antigenic shift – also termed genome reassortment or just reassortment – arises when the HA is exchanged in a virus, for example H1 replaced by H5 resulting in the formation of a mosaic virus. This may happen when a cell is infected by 2 different influenza viruses and their genome segments are exchanged during replication.

This phenomenon of genome reassortment is frequently seen in water birds, especially ducks. Although the birds are seldomly symptomatic after infection, the virus is shed in their faeces for several months.

Neuraminidase

Like HA, neuraminidase (NA or N) is a glycoprotein, which is also found as projections on the surface of the virus. It forms a tetrameric structure with an average molecular weight of 220,000. The NA molecule presents its main part at the outer surface of the cell, spans the lipid layer, and has a small cytoplasmic tail.

NA acts as an enzyme, cleaving sialic acid from the HA molecule, from other NA molecules and from glycoproteins and glycolipids at the cell surface. It also serves as an important antigenic site, and in addition, seems to be necessary for the penetration of the virus through the mucin layer of the respiratory epithelium.

Antigenic drift can also occur in the NA. The NA carries several important amino acid residues which, if they mutate, can lead to resistance against neuraminidase inhibitors. Mutations that have been observed include:

- R292K
- H274Y, R152K, E119V

The letters represent amino acids (R, arginine; K, lysine; H, histidine; Y, tyrosine; E, glutamic acid; V, valine): the former letter is the original amino acid, and the latter the amino acid after mutation occurred.

When the amino acid arginine (R) is replaced by lysine (K) at position 292 of the neuraminidase glycoprotein, complete resistance may result. The mutation of R to K is linked to a single nucleotide exchange of AGA to AAA in the N gene. Position 292 is so significant because mutation may induce resistance not only against the substance oseltamivir, but also against zanamavir and two other new prodrugs.

M2 protein

When the virus particle is taken up in the endosome, the activity of the M2 ion channel is increased so that ions flood into the particle, inducing a low pH. As a result of this, the HA-M1 linkage is disturbed, the particle opens, the fusion peptide within the HA is translocated, and the HA fuses with the inner layer of the endosome membrane. The ribonucleoproteins are liberated into the cytoplasm of the cell and transported to the nucleus, where the complex is disrupted, and viral RNA synthesis is initiated.

The activity of the M2 protein is inhibited by amantadine, rimantadine and related substances.

Possible function of NS1

Human messenger RNA carries a poly-A tail at the 5' end. NS1, with a molecular weight of 26,000, and forms a dimer that inhibits the export of poly-A containing mRNA molecules from the nucleus, thus giving preference to viral RNA which is transported to the ribosome and translated. NS1 might also inhibit splicing of pre-mRNA. In addition, NS1 is probably able to suppress the interferon response in the virus-infected cell leading to unimpaired virus production.

Possible function of NS2

NS2 is a small molecule with a molecular weight of 11,000. In the particle it might be bound to M1 protein. Its function is believed to facilitate the transport of newly synthesised RNPs from the nucleus to the cytoplasm to accelerate virus production.

Replication cycle

Adsorption of the virus

The influenza virus binds to the cell surface by fixing the outer top of the HA to the sialic acid of a cell's glycoproteins and glycolipids. The sialic acid linkage to the penultimate galactose, either alpha 2,3 (in birds) or alpha 2,6 (in humans), determines host specificity. Since sialic acid-presenting carbohydrates are present on several cells of the organism, the binding capacity of the HA explains why multiple cell types in an organism may be infected.

Entry of the virus

After attachment, the virus is taken up by the cell via a clathrin-coated receptor-mediated endocytosis process. When internalised, the clathrin molecules are liberated and the vesicle harbouring the whole virus fuses with endosomes. The contents of the vesicle are usually digested through a stepwise lowering of the pH within the phagosome.

Uncoating of the virus

When a certain level is reached, the lowering of the pH is stopped by the action of the M2 protein which induces the partial liberation of the fusion peptide of the HA. This allows the fusion of the HA with the membrane of the vesicle and liberation of the ribonucleoproteins (RNPs) into the cytoplasm, as described above. The ion influx from the endosome to the virus particle leads to disconnection of the different viral proteins; M1-protein aggregation is disrupted and RNPs no longer adhere to the M1-protein complex. Uncoating is completed within 20-30 min of virus attachment.

Synthesis of viral RNA and viral proteins

The RNPs are transported to the nucleus, where the polymerase complex binds to viral RNA, cleaves viral RNA by its endonuclease activity, and simultaneously leads to elongation. The production of viral RNA is limited by the NP in favour of mRNA. Both are transported to the cytoplasm, where viral proteins are generated at the ribosome. Part of the viral mRNA is spliced by cellular enzymes so that finally viral proteins, such as M1 and NS2, can be synthesised without any further cleavage. Some of the newly synthesised viral proteins are transported to the nucleus where they bind to viral RNA to form RNPs. Other newly synthesised viral proteins are processed in the endoplasmic reticulum and the Golgi apparatus where glycosylation occurs. These modified proteins are transported to the cell membrane where they stick in the lipid bilayer. When they reach a high enough concentration at the plasma membrane, RNPs and M1 proteins aggregate and condense to produce the viral particle. Finally, the particle is extruded from the membrane and will be liberated by the neuraminidase activity.

The time from entry to production of new virus is on average 6 h.

Shedding of the virus and infectivity

Immunohistological pictures show that foci of virus-producing cells are clustered in the mucous layer of the respiratory tract, in the gut and even in endothelial layers, myocardium and brain. Within nasal secretions, millions of virus particles per ml are shed, so that a 0.1 µl aerosol particle contains more than 100 virus particles. A single HID (human infectious dose) of influenza virus might be between 100 and 1,000 particles. At least during the early course of influenza infection, the virus can be found also in the blood and in other body fluids.

Infectivity of influenza virus particles is preserved depending on temperature, pH and salinity of the water, and UV irradiation. At 4°C, the half-life of infectivity is about 2-3 weeks in water. Due to the conformation of the lipid bilayer, survival under normal environmental conditions should be shorter.

Infectivity of the influenza virus particle is easily inactivated by all alcoholic disinfectants, chlorine and aldehydes. As far as is known, temperatures above 70°C will destroy infectivity in a few seconds.

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Chapter 4: Pathogenesis and Immunology

Georg Behrens and Matthias Stoll

Introduction

The influenza virus is notoriously known for its unique ability to cause recurrent epidemics and global pandemics during which acute febrile respiratory illness occurs explosively in all age groups. Two qualities of influenza account for much of the epidemiological spread of the virus. First, is the ability to emerge and circulate in avian or porcine reservoirs by either genetic reassortment or direct transmission and subsequently spread to humans at irregular intervals. Second, is the fast and unpredictable antigenic change of important immune targets once the virus has become established in a human.

A highly contagious virus causing extensive morbidity and major case fatality rates is an archetypal anxiety. Influenza has the potential to create such a scenario. The influenza virus, as a pathogenic agent for humans, has been circulating in the human population since at least the sixteenth century (Cox & Kawaoka 1998) leading to recurrent epidemics of febrile respiratory disease every 1 to 3 years. In addition, each century has seen some pandemics rapidly progressing to involve all parts of the world due to emergence of a novel virus to which the overall population holds no immunity. The characteristics of pandemics include occurrence outside the usual season, extremely rapid transmission with concurrent outbreaks throughout the globe, and high attack rates in all age groups with high mortality rates even in healthy young adults. Given the growing world population and international travel and tourism, impending pandemic influenza outbreaks gain the potential to spread even more rapidly. In order to understand the background of this global epidemic threat more thoroughly, this chapter aims to describe both the pathogenesis of the disease and the contest between the virus and the immune system.

Pathogenesis

The pathogenicity and virulence of the influenza virus is determined by several interacting factors:

a) Host factors:

- Presence of target receptors on host cells
- Availability of enzymes in host cells which are essential for viral entry and replication
- State of immunocompetence of the individual host
- Specific immunity against certain viral epitopes in the individual host and target population
- Ability of the immune system to control the viral replication effectively without causing serious collateral damage for the host by its inflammatory response

b) Viral factors:

- Ability to bind to host cells
- Ability of virus shedding
- Restriction of cytopathogenic effects to allow for an appropriate balance between viral replication and control by the host
- Escape from immunosurveillance by evolution of antigenic variation driven by selective pressure of the immune response
- Escape from immunosurveillance by recombination with different virus strains from zoonotic disease
- Modulation of the immune response to attenuate effective host defense mechanisms

Viral entry: How does the virion enter the host?

The predominant way in which influenza is transmitted is from person to person by aerosols and droplets. Influenza then enters the host through the respiratory tract. In a human lung there are about 300 million terminal sacs, called alveoli, that function in gaseous exchange between inspired air and the blood. The total absorptive area of the human lungs ranges from 80-120 m². The resting ventilation rate in humans is about 6 liters of air per minute, which introduces large numbers of foreign particles and aerosolized droplets potentially containing virus into the lungs. Deposition of foreign particles depends on their size: inhalation of very small particles does not result in absorption through the alveoli or bronchial system. Small droplets with a diameter of approximately 1 to 4 μm precipitate in the small airways. Much larger particles are either not able to enter the respiratory system or are deposited in the upper respiratory tract (Figure 1A).

Numerous host defense mechanisms including mechanical barriers block respiratory tract infection. The respiratory tract is covered with a mucociliary layer consisting of ciliated cells, mucus-secreting cells and glands (Figure 1 B). Foreign particles in the nasal cavity or upper respiratory tract are trapped in mucus, carried back to the throat, and swallowed. From the lower respiratory tract foreign particles are brought up by the ciliary action of epithelial cells. In the alveoli that lack cilia or mucus, macrophages are responsible for destroying particles (Figure 1).

Binding to the host cells

The main targets of the influenza virus are the columnar epithelial cells of the respiratory tract. These cells may be susceptible to infection if the viral receptor is present and functional. Thus, viral receptors are determinants of tropism. However, this simplified model is often insufficient to explain viral tropism since the receptor distribution in the host is generally more widespread than the observed virus tropism.

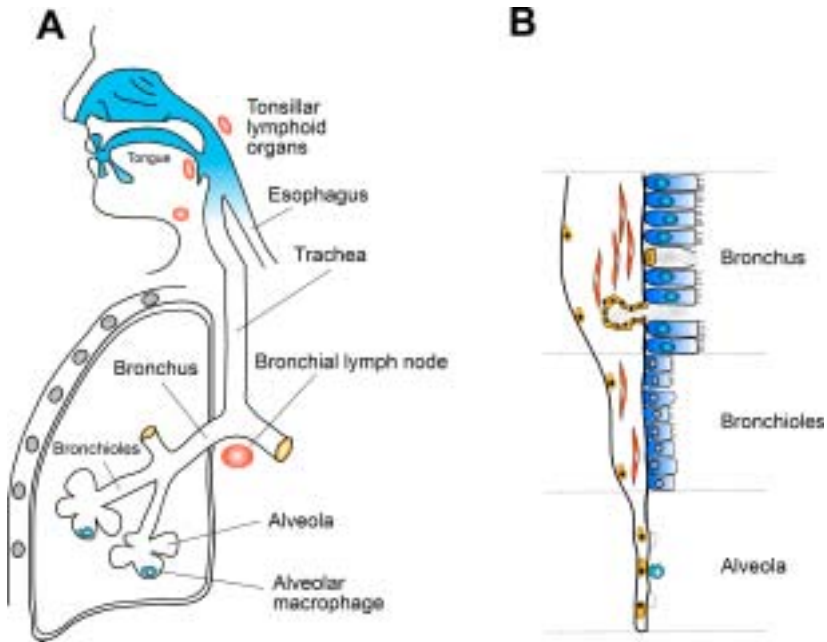


Figure 1. Sites of influenza entry in the respiratory tract. (A) The anatomical and functional structures of the human airways are shown. Influenza first infects the upper airway and the ciliated cells in the bronchus and bronchioli. Resulting clinical syndromes include tracheitis, bronchitis, bronchiolitis, and bronchopneumonia. The adaptive immune response is initiated in lymph nodes along the airways. **(B)** The respiratory epithelia is especially equipped to defend from incoming pathogens by a layer of mucus (bronchus), ciliated cells (bronchus and bronchioli), and alveolar macrophages (alveoli).

In influenza infection, the receptor binding site of viral hemagglutinin (HA) is required for binding to galactose bound sialic acid on the surface of host cells (Weis 1988). Certain areas of the binding site of HA are highly conserved between subtypes of the influenza virus (Daniels 1984). Hosts may prevent the attachment by several mechanisms: (1) specific immune response and secretion of specific IgA antibodies, (2) unspecific mechanisms, such as mucociliary clearance or production of mucoproteins that able to bind to viral hemagglutinin, and (3) genetic diversification of the host receptor (sialic acid), which is highly conserved in the same species, but differs between avian and human receptors (Matrosovich 2000). As a result, the avian virus needs to undergo mutations at the receptor binding site of hemagglutinin to cross the interspecies barrier between birds and humans. In pigs, polymorphisms of sialic acid species and linkage to galactose of both humans and birds are co-expressed in the tissue. Therefore, co-infection with avian and human influenza can occur in pigs and allow genetic reassortment of antigenic properties of both species in the co-infected cells. Recently, it has been shown that certain avian influenza viruses in human and birds are able to bind to different target cells (Matrosovich 2004). This could explain the observation of several cases since the end of the 1990s with transmission of avian influenza directly from poultry to humans. H5N1 and some other subtypes of influenza A virus are able to bind to receptors in the human eye (Olofson 2005).

As essential as the binding of the influenza virus is its cleavage from the binding site at the host cell. Cleavage is the functional role of viral neuraminidase (Chen 1998). The virulence of the influenza virus depends on the compatibility of neuraminidase with hemagglutinin. A virulent virus which has undergone mutations in the hemagglutinin needs compensatory mutations in the neuraminidase to maintain its virulence (Baigent & McCauley 2003, Hulse 2004). As a consequence, viral fitness and virulence were found to be reduced in influenza viruses resistant to neuraminidase inhibitors (Yen 2005).

Once the cell membrane and the virus have been closely juxtaposed by virus-receptor interaction, the complex is endocytosed. Importing H^+ ions into the late endocytic vesicles as a physiologic event then acidifies the interior. Upon acidification, the viral HA undergoes a conformational rearrangement that produces a fusogenic protein. The loop region of the HA becomes a coiled coil eventually bringing the viral and endosomal membranes closer so that fusion can occur. To allow release of viral RNA into the cytoplasm, the H^+ ions in the acidic endosome are pumped into the virion interior by the M2 ion channel. As a result, viral RNA dissociates from M1 by disrupting the low pH-sensitive interaction between the M1 and ribonuclein complex after fusion of the viral and endosomal membranes. The viral RNA is then imported in an ATP-dependent manner into the nucleus for transcription and translation (Flint 2004).

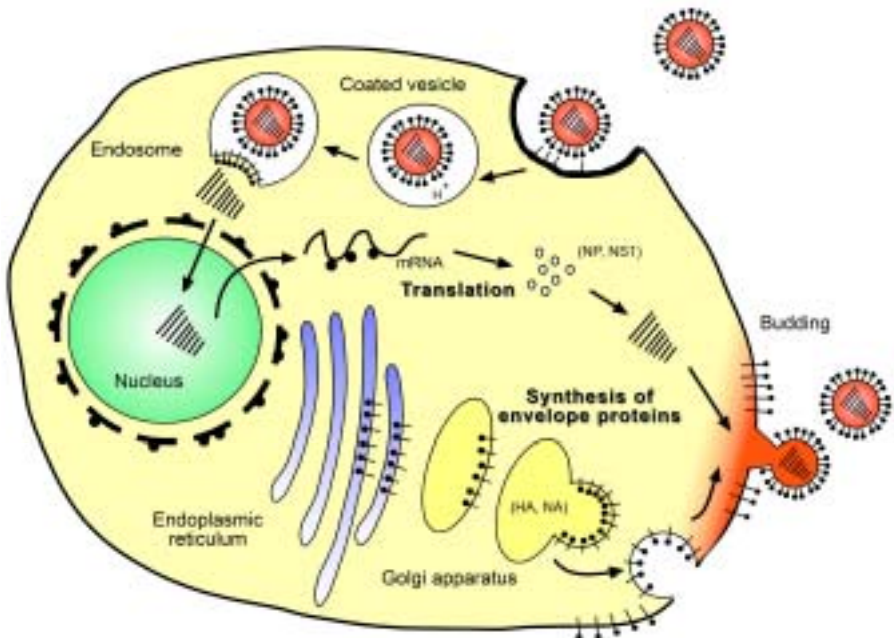


Figure 2: Replication cycle of influenza A virus. Binding and entry of the virus, fusion with endosomal membrane and release of viral RNA, replication within the nucleus, synthesis of structural and envelope proteins, budding and release of virions capable of infecting neighboring epithelial cells (Modified from Cox & Kawaoka 1997)

Where does the primary replication occur?

Cellular proteases are often required to cleave viral proteins to form the mature infectious virus particle. Thus, additional factors to entry receptors can determine the site of viral replication. In humans, the replication of the influenza virus is generally restricted to the epithelial cells of the upper and lower respiratory tract. This is because of the limited expression of serine protease, trypsin, secreted by non-ciliated Clara cells of the bronchial epithelia. The purified enzyme cleaves the polypeptide HA chain precursor HA0 of extracellular particles and activates HA in virions rendering them infectious. Some highly virulent avian influenza strains, however, contain genetic insertions at the cleavage site of HA leading to processing by ubiquitous proteases. This may cause altered tropism and additional sites of replication in animals and humans (Gamblin 2004). Tissue tropism of avian influenza (H5N1) in humans is not well defined. In one case, viral RNA was detected in lung, intestine, and spleen by a reverse transcription polymerase chain reaction but positive-stranded viral RNA, indicating virus replication, was confined exclusively to the lung and intestine (Uiprasertkul 2005). Thus, H5N1 viral replication in humans may be restricted to the respiratory and intestinal tract in contrast to disseminated infections documented in other mammals and birds.

How does the infection spread in the host?

Once influenza has efficiently infected respiratory epithelial cells, replication occurs within hours and numerous virions are produced. Infectious particles are preferentially released from the apical plasma membrane of epithelial cells into the airways by a process called budding. This favors the swift spread of the virus within the lungs due to the rapid infection of neighboring cells.

Alterations in the HA cleavage site by naturally occurring mutants can dramatically influence the tropism and pathogenicity of influenza. As a result, it can be recognized by other cellular proteases. This would explain why many of the individuals infected with avian influenza (H5N1) in Hong Kong had gastrointestinal, hepatic, and renal, as well as respiratory symptoms and why viruses from these patients were neurovirulent in mice (Park 2002). Whether these symptoms result from hematogenic spread or reflect non-pulmonary means of viral entry into the host remains unclear. However, mutation in NA may also, in part, explain the pantropic nature of influenza. For example, the laboratory-derived WSN/33 strain of influenza, a variant of the first human influenza virus ever isolated, unlike most human influenza strains, can replicate *in vitro* in the absence of added trypsin. In this virus an in-frame deletion that removes the glycosylation site at residue 46 of NA allows neuraminidase to bind and sequester plasminogen. This leads to higher local concentrations of this ubiquitous protease precursor and thus to increased cleavage of the HA. These findings suggest a means by which influenza A viruses, and perhaps other viruses as well, could become highly pathogenic in humans. (Goto & Kawakita 1998). Interestingly, studies with the genetically reconstructed 1918 Spanish influenza pandemic virus (H1N1) revealed additional mechanisms of NA-mediated HA cleavability that may be relevant to the replication and virulence of that virus (Tumpey 2005).

Finally, animal studies have revealed that the site of inoculation can determine the pathway of spread of the influenza virus in the host. For example, the neurotropic

NWS strain disseminates to the brain by hematogenous spread when given intraperitoneally but reaches the central nervous system via the sensory neurons when the virus inoculum is placed in the nose (Flint 2004). The latter has been demonstrated with the Hong Kong H5N1 virus as well (Park 2002).

What is the initial host response?

Although a frequent disease, the specific inflammatory patterns or regulation of immune response and the pathogenesis of cytopathic effects in human influenza is incompletely understood. Most evidence comes from animal studies, where avian influenza is a disseminated disease. The pathophysiology of such models, however, may profoundly differ from that in humans.

Cytokines and fever

A central question is how an infection essentially localized to the respiratory tract can produce such severe constitutional symptoms. As in many other infectious diseases, it is the unspecific and adaptive immune response that contributes substantially to the clinical signs and symptoms in influenza and finally to the control of infection. These immune mechanisms can lead to both localized as well as systemic effects. Cytokines, rapidly produced after infection by epithelial and immune cells of the respiratory mucosa, are local hormones that activate cells, especially within the immune system. Chemokines are a subset of cytokines that act as chemoattractants for cells of the immune system. For example, influenza infection induces in human plasmacytoid and myeloid dendritic cells a chemokine secretion program which allows for a coordinated attraction of the different immune effectors (Piqueras 2005, Schmitz 2005). The most important cytokines serve as endogenous pyrogens and are involved in the pathogenesis of fever: IL-1 α/β , TNF α/β , IL-6, interferon (IFN) α/γ , IL-8, and macrophage inflammatory protein (MIP)-1 α .

Most of these cytokines have been detected in nasopharyngeal washes of humans who have been experimentally or naturally infected with influenza (Brydon 2005). It is proposed that these cytokines, produced locally or systemically following interaction of exogenous pyrogens (e.g. influenza) with phagocytes, reach the central nervous system. There is a small area in the hypothalamus, called the Organum vasculosum laminae terminalis, which has a reduced blood-brain-barrier and allows the passage of pyrogens. At this site, in a dose-dependent manner, they induce the production of prostaglandins and especially prostaglandin E₂. These mediators increase the thermostatic set point and trigger complex thermoregulatory mechanisms to increase body temperature. The fact that none of the cytokines mentioned above correlated with the severity of disease in influenza infection, argues in favor of their pleiotropy and cross-talk amongst signaling pathways.

The relevance of cytokines may also differ between influenza strains or individuals. Influenza infections with the Hong Kong H5N1 strain from 1997 have been proposed to potently induce pro-inflammatory cytokines (particularly TNF α) by NS gene products (Cheung 2002, Lipatov 2005, Chan 2005). Studies aimed to identify other virion components that induce cytokine release revealed that double-stranded (ds) RNA, either from lungs of infected mice or synthetically derived from influenza, were pyrogenic when injected into the CNS-ventricle of mice. Such dsRNA is released from infected cells when they die and thus may stimulate cytokine production. Recent studies indicate that dsRNA-sensing Toll-like receptor (TLR) 3 is ex-

pressed on pulmonary epithelial cells and that TLR3 contributes directly to the immune response of respiratory epithelial cells (Guillot 2005, Akira & Takeda 2004). Interestingly, in humans the initiation of an innate immune response against influenza appears to be at least as dependent on sensing single stranded RNA via TLR 8 than on detecting dsDNA by TLR 3. Virus particles can also be pyrogenic, as viro-somes depleted of RNA but including viral lipid, hemagglutinin, and neuraminidase may induce fever. Individual virion components were, however, not pyrogenic probably explaining why whole virus vaccines can produce influenza-like symptoms while subunit vaccines do not (Brydon 2005).

Respiratory symptoms

Hyperreactivity of the bronchial system (Utell 1980, Little 1978), obstruction predominantly of small airways (Hall 1976) and impaired diffusion capacity (Horner 1973) is common in influenza infection. Hyperreactivity and broncho-obstruction may persist for a prolonged period, especially in allergic disease (Kondo & Abe 1991), and might be a result of a pro-inflammatory cytokine profile which interferes with the ability to induce tolerance to aerosolized allergens (Tsitoura 2000).

In human influenza infection, severe alveolar inflammation presenting as primary viral pneumonia, is rare. It usually presents with extended inflammation of both lower and upper respiratory tracts with loss of ciliated cells, and imposed hyperemic or hemorrhagic areas on hyaline membranes and infiltrates of neutrophils and mononuclear cells (Yeldandi & Colby 1994).

In contrast to primary viral pneumonia, bacterial superinfection is common in human influenza and causes serious morbidity and mortality predominantly in elderly adults. Several factors have been identified, which could explain the increased risk for bacterial infection of the respiratory tract, including damage of columnar epithelial cells with disruption of the epithelial cell barrier (Mori 1995), decreased mucociliary clearance (Levandovski 1985), enhancement of bacterial adherence (McCullers 2002), and functional alteration of neutrophils (Abramson 1986, Cassidy 1988).

Cytopathic effects

Human influenza leads to complex cytopathic effects, predominantly at the columnar epithelial cells in the respiratory tract, that result in acute disease of lung and airways. Infection and viral replication of the influenza virus in the respiratory tract leads to cell damage induced by downregulation of host cell protein synthesis (Katze 1986, Sanz-Esquerro 1995) and apoptosis (Wiley 2001a). The latter, also called programmed cell death, is a series of defined cellular events that eventually results in the efficient removal of the cell and its contents. Apoptosis can be triggered by different mechanisms and is characterized by several morphological changes, including cytoskeleton disruption, condensation of cytoplasm and chromatin, loss of mitochondrial function, DNA fragmentation, and ultimately the formation of small membrane bound particles known as apoptotic bodies, which are cleared by phagocytic cells such as macrophages and dendritic cells.

The influenza virus-induced apoptosis is mediated by both Fas-mediated mechanisms and Fas-independent signals, such as the formation of FADD/caspase-8 complex by protein kinase R (PKR), which initiates a caspase cascade. PKR is a key regulatory component in many apoptotic pathways and is induced by IFN and acti-

vated by dsDNA (Brydon 2005). As a third pathway to apoptosis, influenza activates transforming growth factor (TGF)- β via viral neuraminidase. NA can activate latent TGF- β on the cell surface by facilitating cleavage of TGF- β into its active form. TGF- β initiates a signaling cascade leading to the activation of the c-Jun N-terminal kinase (JNK) or stress activated protein kinase (SAPK), resulting in the activation of transcription factors and upregulation of pro-apoptotic gene expression. This pathway, together with the effects on the mitochondrial membrane stability of a small protein, encoded by an alternative +1 reading frame in the PB1 protein (Chen 2001), has been implicated in the apoptosis of lymphocytes and could explain the lymphopenia observed during acute infection.

Lung tissue injury following infection with the influenza virus has been associated with cellular oxidative stress, generation of reactive oxygen species (ROS), and the induction of nitric oxide synthetase-2, which leads to the formation of toxic reactive nitrogen intermediates. Anti-oxidants, however, had little effect on apoptosis in bronchiolar cell lines *in vitro*.

Symptoms of H5N1 infections

Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus. To date, all outbreaks of the highly pathogenic form have been caused by influenza A viruses of subtypes H5 and H7. It is currently unknown whether avian influenza in humans (H5N1) has the same cytopathic effects as mentioned above. Only a few studies in severe or fatal cases have been performed. However, asymptomatic or mild symptomatic disease is possible (Buxton Bridges 2000, Katz 1999) and its incidence may be underestimated.

The most common initial symptoms of H5N1 influenza in humans were high fever, and, in those patients referred to a hospital, pneumonia, pharyngitis, intestinal symptoms, conjunctivitis, and acute encephalitis (Yuen 1998, Tran 2004, Yuen & Wong 2005). Adult patients with initial signs of pneumonia often progressed to an ARDS-like disease. In fatal cases of H5N1-influenza, reactive hemophagocytic syndrome has been described as a prominent feature. Beyond pulmonary disease with organizing diffuse alveolar damage and interstitial fibrosis, extrapulmonary involvement has been described as extensive hepatic central lobular necrosis, acute renal tubular necrosis and lymphoid depletion (To 2001), although there was no virus found on isolation, reverse transcription polymerase chain reaction and immunostaining respectively. Soluble interleukin-2 receptor, interleukin-6 and interferon-gamma were increased. In addition, tumor necrosis factor-alpha mRNA was seen in lung tissue in other cases with H5N1 influenza in humans (Uiprasertkul 2005).

In comparison to human H1N1 viruses (Hayden 1998), the Hong Kong H5N1 strain from 1997 has been proposed to potentially induce pro-inflammatory cytokines including IL-10, IFN β , RANTES, IL-6 and particularly TNF α by NS gene products (Cheung 2002, Lipatov 2005, Chan 2005). The authors of these studies postulated that in a fatal human infection with the avian H5N1 subtype, initial virus replication in the respiratory tract triggers hypercytokinemia complicated by a reactive hemophagocytic syndrome, which might be a different pathogenesis of influenza A H5N1 infection from that of usual human subtypes (To 2001). Bacterial superinfection has not been found in fatal cases of H5N1 avian influenza (To 2001). This observation might be a bias of the early fatal outcome of these most severe cases, which hypothetically did not allow for the development of superinfection.

How is influenza transmitted to others?

Respiratory transmission depends on the production of virus-containing airborne particles and aerosols. Aerosols are produced during speaking and normal breathing. Shedding from the nasal cavity requires sneezing and is much more effective if the infection produces a nasal secretion. A sneeze produces up to 20,000 droplets in contrast to several hundred expelled by coughing. The largest droplets fall to the ground within a few meters. The remaining droplets travel a distance dependent on their size. Droplets measuring 1-4 μm in diameter may remain suspended for a long time and reach the lower respiratory tract. Experimental transmission of influenza in volunteers showed that bronchial inhalation of small droplets is superior in comparison to inoculation of large droplets into the upper respiratory tract or conjunctiva (Alford 1966, Little 1979, Bridges 2003). If the virus replicates early during the course of infection in the lower respiratory tract, this would result in smaller droplets with higher viral load and higher infectivity, because specific immunosurveillance is still not established. Transmission of H5N1 from animal to human may occur in a different way by direct (and indirect) contact to infected poultry.

High attack rates are necessary to result in an epidemic outbreak of influenza A. Therefore winter epidemics in Europe and North America may be explained by closer contacts and stay in less ventilated rooms. Influenza virus is well adapted: for unknown reasons its ability to survive is best in lower relative humidity and at lower environmental temperatures (Hemmes 1960). Avian influenza (H5N1) might be less adapted to droplet transmission: the incubation period is longer (Chotpitaya-sunondh 2005), theoretically resulting in less simultaneous onset in many persons during an epidemic. Intestinal replication and symptoms precede respiratory manifestations by up to one week (Apisarnthanarak 2004), allowing onset of specific immune response before spread by infectious droplets can evolve. As a consequence, nasopharyngeal replication in avian influenza is less than in human influenza (Peiris 2004) but viral replication is prolonged (Beigel 2005). Until now transmission of H5N1 between humans has been rare (Buxton Bridges 2000, Ungchusak 2005) and rather inefficient. In conclusion, avian influenza virus (H5N1) presumably requires several passages to enable human-to-human transmission and to finally reach an infectivity rate which is effective enough to generate an epidemic or pandemic.

Immunology

Influenza causes an acute infection of the host and initiates a cascade of immune reactions activating almost all parts of the immune defense system. Most of the initial innate response, including cytokine release ($\text{IFN}\alpha/\beta$), influx of neutrophil granulocytes or natural killer cells (Mandelboim 2001, Achdount 2003), and cell activation, is responsible for the acute onset of the clinical symptoms (see above). Innate immunity is an essential prerequisite for the adaptive immune response, firstly, to limit the initial viral replication and antigen load, and secondly, because the antigen-specific lymphocytes of the adaptive immune response are activated by co-stimulatory molecules that are induced on cells of the innate immune system during their interaction with viruses (Figure 3). Influenza viruses, however, encode

in the non-structural protein 1 (NS1) mechanisms to evade and antagonize the IFN α/β response. NS1 is likely to sequester viral dsRNA which prevents recognition of this dangerous molecule by cellular sensors which would otherwise trigger IFN α/β release (Garcia-Sastre 1998, Garcia-Sastre 2005).

The adaptive immune response requires some days to be effective but then helps to contain the viral spread, to eradicate the virus, and finally to establish a memory response resulting in a long-lived resistance to re-infection with homologous virus. Cross-protection within a subtype of influenza has only rarely been observed and infections essentially induce no protection across subtypes or between types A and B (Treanor 2005). Influenza infection induces both systemic and local antibody (humoral immunity), as well as cytotoxic T cell responses (cellular immunity), each of which is important in recovery from acute infection and resistance to reinfection.

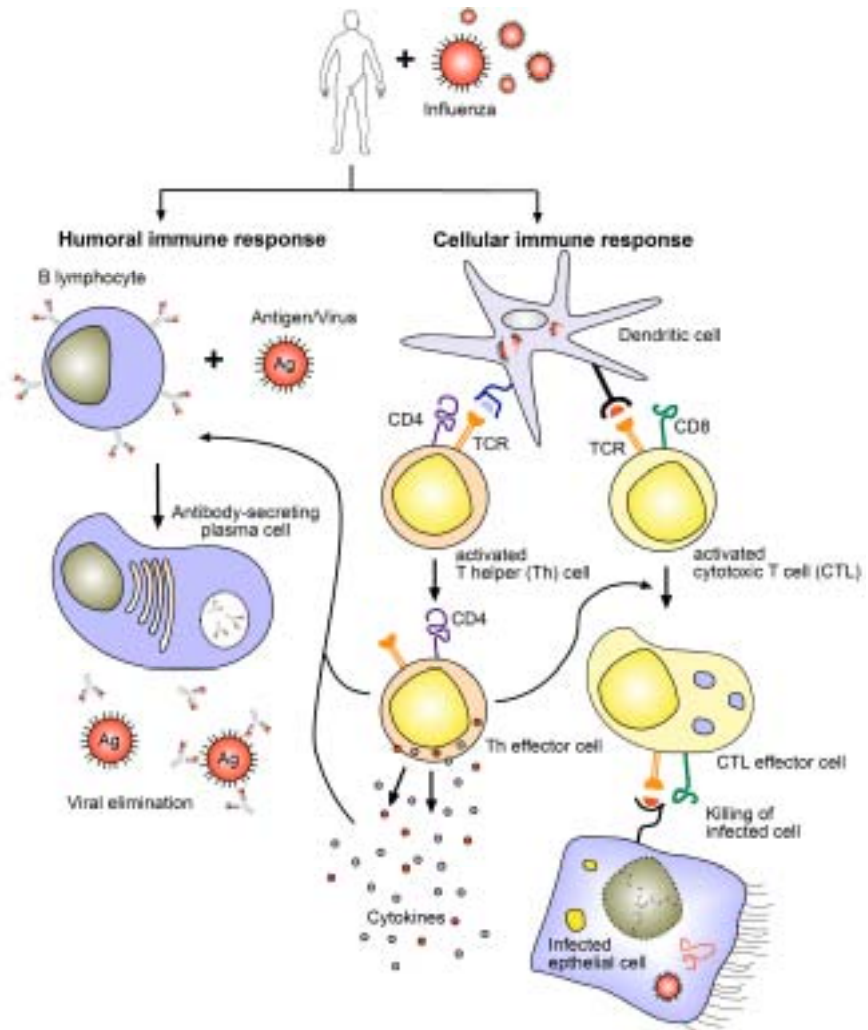


Figure 3. The humoral and cell-mediated immune response to influenza virus infection. The humoral branch of the immune system comprises B-lymphocytes (*left*), which after interaction with influenza differentiate into antibody-secreting plasma cells. The cellular response (*right*) starts with antigen presentation via MHC I (black) and II (blue) molecules by dendritic cells, which then leads to activation, proliferation and differentiation of antigen-specific T cells (CD4 or CD8). These cells gain effector cell function to either help directly, release cytokines, or mediate cytotoxicity following recognition of antigen (Adapted from Flint 2004). Not shown is the formation of a cellular memory immune response and the various forms of innate immunity induced by influenza.

The humoral immune response

Antibodies (e.g. IgG, IgA) are produced by plasma cells which are the final stage of B cell development, requiring that the B cells have recognized antigen and been stimulated by CD4 T cells and T cell-derived cytokines (Figure 3). Unlike T cells, B cells can recognize antigen in its native form. The antigen specificity arises from random rearrangements of genes coding for the hypervariable region of immunoglobulins in the cells, whilst still in the bone marrow. The naïve B cells then enter the circulation and travel via the blood stream and lymphatics through tissue and lymphoid organs. In the lymph nodes, naïve B cells recognize cognate antigen by their surface antibodies, become activated, switch from IgM to IgG production (class-switch), increase their immunoglobulin specificity and affinity, and differentiate into plasma cells or memory B cells as the cell continues to divide in the presence of cytokines. While IgA is transported across the mucosal epithelium of the upper airway, where it serves to neutralize and clear viral infection, IgG is primarily responsible for the protection of the lower respiratory tract (Palladino 1995, Renegar 2004).

Influenza infection results in the systemic production of antibody to both influenza glycoproteins HA and NA, as well as M and NP proteins. For example, HA-specific immunoglobulins, including IgM, IgA and IgG, appear within 2 weeks of virus inoculation. The development of anti-NA parallels that of hemagglutinin-inhibiting antibodies. The peak in antibody titers are seen between 4-7 weeks after infection, and are followed by a steady decline. Antibodies remain detectable for years after infection even without re-exposure. The anti-HA antibody protects against both disease and infection with homologous virus, and the induction of neutralizing antibodies is one of the main goals of immunization with vaccines. Serum HA-inhibiting titers of 1:40 or greater, or serum neutralizing titers of 1:8 or greater, are supposed to protect against infection. Higher levels of antibody are required for complete protection in older individuals (Treanor 2005).

In contrast to anti-HA antibody, anti-NA antibody does not neutralize virus infectivity, but instead reduces the efficient release of virus from infected cells (Johansson 1989). This is because neuraminidase cleaves the cellular-receptor sialic acid residues to which the newly formed particles are attached. Anti-NA antibody can protect against the disease and results in decreased virus shedding and severity of symptoms. Similar effects have been proposed for antibodies against M2 protein of influenza A, although in general, antibodies against internal antigens are non-neutralizing, disappear more rapidly and do not appear to play a role in protective immunity.

The mucosal immune response against influenza, as measured in nasal secretions, is characterized by the presence of IgA and IgG₁ against HA. The mucosal anti-HA IgG levels correlate well with the respective serum levels, indicating passive diffusion from the systemic compartment, whereas IgA is produced locally. Studies suggest that resistance to reinfection is predominantly mediated by locally produced HA-specific IgA, although IgG might be relevant as well (Renegar 2004). Either mucosal or systemic antibody alone can be protective if present in sufficient concentrations, and optimal protection occurs when both serum and nasal antibodies are present (Treanor 2005). Antibodies act in immunity against influenza by neutralization of the virus or lysis of infected cells via complement or antibody-dependent cellular toxicity.

Hosts that survive an acute virus infection and clear the virus are in general immune to infections by the same virus. Nevertheless, acute infections caused by influenza virus occur repeatedly, despite active immune clearance. This is because influenza displays a structural plasticity as it can tolerate many amino acid substitutions in its structural proteins without losing its infectivity. As an example, the sialic acid receptor-binding molecule HA, responsible for entry of the virus into the target cell, is also a main target for neutralizing antibodies and cytotoxic T lymphocytes, which exhibit a continuous immunological pressure. This immune selection or diversity, which arises from copying errors, results in slight variations of HA over time that permit the virus to evade human immune responses (antigenic drift). These changes are the reason for the annual epidemic spread of influenza and require new vaccines to be formulated before each annual epidemic. In contrast, antigenic shift is a major change in the surface protein of a virion, as genes encoding completely new surface proteins arise after recombination or reassortment of genomes or genome segments. Antigenic drift is possible every time a genome replicates. In contrast, antigenic shift can only occur under certain circumstances, is relatively rare and the likely reason for pandemics.

The cellular immune response

Dendritic cells have been shown to play a central role in initiating and driving T lymphocyte responses. They are a sparsely distributed, migratory group of bone-marrow derived leukocytes that are specialized for the uptake, transport, processing and presentation of antigens to T cells (Figure 3). The basic paradigm is that lung-resident dendritic cells acquire antigen from the invading pathogen, become activated, and subsequently travel to the local draining lymph nodes (Legge & Braciale 2003). The antigenic sample is processed and fixed on the dendritic cell surface as peptides which are presented by major histocompatibility complex (MHC) molecules (Silver 1992). In the lymph nodes, the now mature dendritic cells efficiently trigger an immune response by any T cell with a receptor that is specific for the foreign-peptide-MHC complex on the dendritic cell surface (Shortman & Liu 2002). Endogenous antigens from the viral infection of dendritic cells are processed and presented to CD8 T cells on MHC I molecules. Exogenous antigens are presented via MHC II molecules to CD4 T lymphocytes. Alternatively, dendritic cells may present antigens they have acquired by uptake from infected cells, or transfer antigen to neighboring dendritic cells in the lymph node which then initiate a CD8 T cell response by a process called cross-presentation (Belz 2004, Heath 2004, Wilson 2006). The newly activated T cells acquire effector cell functions and migrate

to the site of infection in the lung where they mediate their antiviral activities (Figure 3).

Following recovery from an infection, a state of immunological memory ensues in which the individual is better able to control a subsequent infection with the same pathogen (Ahmed & Gray 1996). Memory is maintained by antigen-specific T cells that persist at increased frequencies, have reduced requirements for co-stimulatory signals in comparison to naïve T cells, and respond quickly to antigenic re-stimulation (Woodland & Scott 2005). There is also evidence in favor of a site specific accumulation of influenza-specific CD8 memory T cells in human lungs for the immediate immunological protection against pulmonary re-infection (de Bree 2005, Wiley 2001b). During influenza infection, both CD4 and CD8 memory T cell subsets respond to, and mediate control of an influenza virus re-infection, which is in contrast to the primary infection where viral clearance depends on CD8 T lymphocytes (Woodland 2003).

Another important feature, in for example influenza infection, is that CD4 T lymphocytes help B lymphocytes to generate anti-HA and anti-NA antibodies (Figure 3). The epitopes in HA recognized by the CD4 T helper cells are distinct from those recognized by antibodies. T helper (Th) cells may also promote the generation of virus-specific CD8 cytotoxic T lymphocytes. Th cells can be further subdivided into at least Th1 and Th2 cells, based on the type of cytokines they produce. In mice, influenza infection induces a strong Th1 response, but Th2 cytokines (IL-4, IL-5, IL-6, IL-10) have also been found in the lungs of infected animals. Some evidence indicates that protective immunity is mediated by Th1-like responses. In influenza infection, CD8 cytotoxic T lymphocytes (CTL) recognize epitopes from HA or internal proteins M, NP, or PB2 presented on MHC class I molecules (Trenor 2005). Depending on their antigen specificity, CTLs may be subtype-specific or, in case they recognize internal antigens, broadly cross-reactive with influenza A. Animal experiments using adoptive transfer of CTLs revealed their proliferation and migration pattern during infection (Lawrence & Braciale 2004, Lawrence 2005) and their potential in mediating recovery from influenza infection. They are, however, not absolutely required for the control of influenza.

T lymphocyte responses in humans peak at about day 14 post infection and levels of influenza-specific CTLs correlate with a reduction in the duration and level of virus replication in adults. Memory CD8 T cells may play a role in ameliorating the severity of disease and facilitating recovery upon reinfection. Recent studies in animals suggest that the recall response in lungs is comprised of several distinct phases that are temporally and anatomically separated. The first phase is mediated by memory T cells that are resident in the lung airways (Woodland & Radall 2004). Importantly, these cells are able to respond to the first signs of infection when the viral load is still very low. While unable to proliferate in response to infection due to the constraints of the airway environment, they can produce cytokines that may limit viral replication and spread in the epithelium. The second phase of the response is mediated by memory T cells that are rapidly recruited to the airways in the first few days of the response. The third stage is the antigen-driven expansion of memory T cells that occurs in the secondary lymphoid organs. These memory cells proliferate for several days in the lymphoid organs and are only recruited to the lung airways after about 5 days of infection (Woodland & Randall 2004). Whether these complex models generated from animal experiments apply to the situation in

humans is unclear. It will be essential, however, to better understand the types of immune response and the generation and maintenance of an effective memory T cell response during influenza infection in order to improve future vaccine strategies.

Conclusion

We have seen how influenza virus infection leads to the acute development of a febrile respiratory illness. The pathogenesis is characterized by the rapid replication and distribution of the virus within the lungs, causing local and systemic inflammation and cytokine release. These events, together with the adaptive immune response, help to reduce the viral burden, to eliminate the virus, and to trigger disease recovery. The humoral and cellular immune responses, provoked by infection or vaccination, provide individuals and populations with long-lasting protective immunity against related viral strains. Influenza, however, can undermine this infection- or vaccine-derived immunity by means of antigenic shift and drift, resulting in epidemic and pandemic outbreaks. Technical improvements, including genetic and functional studies, will help to gain a deeper insight into the pathogenesis of historic and currently circulating virulent influenza strains. This knowledge and an advanced understanding about the viral immune defense mechanisms in the human lung will hopefully facilitate the development of better treatment options and more effective vaccines to be distributed worldwide against present and future influenza virus variants.

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106 Pathogenesis and Immunology

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Chapter 5: Pandemic Preparedness

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Introduction

Previous Influenza Pandemics

Three influenza pandemics (worldwide epidemics) are known to have occurred, all caused by influenza A viruses. When a significant change in at least one of the influenza A virus surface proteins haemagglutinin and neuraminidase occurs spontaneously, nobody has immunity to this entirely new virus. If the virus also achieves efficient human-to-human transmission and has the ability to replicate in humans causing serious illness, a pandemic can occur. This happened in 1918 (the “Spanish flu”, caused by a H1N1 subtype), in 1957 (the “Asian flu” caused by a H2N2 subtype) and in 1968 (the “Hong Kong flu”, caused by a H3N2 subtype). Conservative estimates suggested that the mortality from the 1918 pandemic was 20 to 40 million. However, recent studies from Africa and Asia suggest that the number of victims worldwide might have been closer to 50–100 million (Johnson 2002).

Influenza experts have estimated that in industrialised countries alone, the next influenza pandemic may result in up to 130 million outpatient visits, 2 million hospital admissions and 650,000 deaths over two years. The impact is likely to be even greater in developing countries (WHO 2004). A 1918-type influenza pandemic today is projected to cause 180–360 million deaths globally (Osterholm 2005).

H5N1 Pandemic Threat

So far (January 2006), nine countries in the Far East have reported poultry outbreaks of a highly pathogenic H5N1 avian influenza virus: the Republic of Korea, Vietnam, Japan, Thailand, Cambodia, Laos, Indonesia, China, and Malaysia. The outbreaks in Japan, Malaysia, and the Republic of Korea were successfully controlled, but the virus seems to have become endemic in several of the affected countries. The Southeast Asian outbreaks resulted in the death or destruction of more than 150 million birds and had severe consequences for agriculture, most especially for the many rural farmers who depend on small backyard flocks for income and food.

The recent outbreaks of the same virus strain in birds in Russia, Kazakhstan, Turkey, Romania, and Croatia provide evidence that it has spread beyond the initial focus (WHO 2005a, WHO 2005b).

Human cases of avian influenza A (H5N1), most of which have been linked to direct contact with diseased or dead poultry in rural areas, have been confirmed in six countries: Vietnam, Thailand, Cambodia, Indonesia, China, and Turkey (see Table 1). The figures for confirmed human cases of avian influenza A (H5N1) infection reported to the WHO are regularly updated on the WHO webpage (WHO 2005c).

Table 1. Cumulative Number of Confirmed Human Cases of Avian Influenza A / (H5N1) Reported to the WHO up until January 25, 2006 (WHO 2005c) *

	Cases**	Deaths
Vietnam	93	42
Thailand	22	14
Cambodia	4	4
Indonesia	19	14
China	10	7
Turkey	4	2
Total	152	83

* WHO reports only laboratory-confirmed cases.

** Total number of cases includes number of deaths

Recent research suggests that the 1918 virus might not have been a reassortant virus (like those of the 1957 and 1968 pandemics), but more likely an entirely avian-type virus that adapted to humans (Taubenberger 2005). There is some evidence that the high pathogenicity of the 1918 virus was related to its emergence as a human-adapted avian influenza virus. The intriguing similarity in a number of changes in the polymerase proteins of both the 1918 strain and in the recently circulating, highly pathogenic strains of H5N1 avian viruses that have caused fatalities in humans (Taubenberger 2005), is reason for concern.

Considering that H5N1 is antigenically new, is highly pathogenic in humans and that it may acquire the ability to be efficiently transmitted from human to human, the World Health Organisation reiterated its 1997 call for all countries to prepare for the next pandemic, which it termed “inevitable and possibly imminent” (BWHO 2004), and updated its own pandemic preparedness plan in April 2005 (WHO 2005d).

Influenza Pandemic Preparedness

Planning is essential for reducing or slowing transmission of a pandemic influenza strain and for decreasing or at least spreading out the number of cases, hospitalisations and deaths over time. Preparedness will help to maintain essential services and to reduce the economic and social impact of a pandemic (WHO 2004).

Epidemiological models indicate that a pandemic would have the greatest impact on the poorest countries, as a result of limited surveillance and healthcare resources, as well as the general poor health and nutritional status of the population (WHO 2004).

Pandemic Phases

In order to define the sequence of actions during certain key events, the WHO Global Influenza Preparedness Plan (WHO 2005d) distinguishes different phases. Each phase is associated with international and national public health actions. The national actions to be taken during each phase are further subdivided according to the national epidemiological situation. The WHO strongly recommends that countries consider the national actions proposed in the WHO Global Influenza Preparedness Plan when developing or updating a national plan. A summary of these new phases is presented in Table 2. The world is presently (January 2006) in phase 3, as

a new influenza virus subtype is causing disease in humans, but is not yet spreading efficiently and sustainably among humans.

Table 2. Phases according to the WHO Global Influenza Preparedness Plan of 2005 (based on WHO 2005d).

Period/ Phase	Event
Interpandemic Period	
Phase 1	No new influenza virus subtypes have been detected in humans. An influenza virus subtype that has caused human infection may be present in animals. If present in animals, the risk ^a of human infection or disease is considered to be low.
Phase 2	No new influenza virus subtypes have been detected in humans. However, a circulating animal influenza virus subtype poses a substantial risk ^a of human disease.
Pandemic Alert Period	
Phase 3	Human infection(s) with a new subtype, but no human-to-human spread, or at most rare instances of spread to a close contact.
Phase 4	Small cluster(s) with limited human-to-human transmission but spread is highly localised, suggesting that the virus is not well adapted to humans ^b .
Phase 5	Larger cluster(s) but human-to-human spread still localised, suggesting that the virus is becoming increasingly better adapted to humans, but may not yet be fully transmissible (substantial pandemic risk) ^b .
Pandemic period	
Phase 6	Pandemic phase: increased and sustained transmission in the general population ^b .
Postpandemic period	
	Return to interpandemic period.

a. The distinction between *phase 1* and *phase 2* is based on the risk of human infection or disease resulting from circulating strains in animals. The distinction would be based on various factors and their relative importance according to current scientific knowledge. Factors may include: pathogenicity in animals and humans; occurrence in domesticated animals and live-stock or only in wildlife; whether the virus is enzootic or epizootic, geographically localised or widespread; other information from the viral genome; and/or other scientific information.

b. The distinction between *phase 3*, *phase 4* and *phase 5* is based on an assessment of the risk of a pandemic. Various factors and their relative importance according to current scientific knowledge may be considered. Factors may include: rate of transmission; geographical location and spread; severity of illness; presence of genes from human strains (if derived from an animal strain); other information from the viral genome; and/or other scientific information.

Inter-Pandemic Period and Pandemic Alert Period Surveillance

Surveillance has been defined as “an ongoing systematic collection, analysis, and interpretation of outcome-specific data for use in the planning, implementation, and evaluation of public health practices”, and not merely collection of data (Flahault

1998). Thus, a timely, representative and efficient surveillance system is the cornerstone of control of epidemic-prone communicable diseases (PPHSN 2004).

In order to be able to detect an unusual cluster or number of cases of illness that may be due to a new influenza virus, it is essential for every country to have an early warning system for human disease. By participating in the Global Influenza Surveillance Network, a country contributes to the detection of influenza viruses with pandemic potential. The type of surveillance will depend on whether a potential pandemic strain of influenza virus has first been recognised in domestic animals, in wild animals or in humans, and in which geographical area the new strain is known or expected to be circulating (WHO 2005e).

Surveillance should lead to action. Before setting surveillance priorities, countries should define the objectives of surveillance. Speed of laboratory confirmation will affect the rapidity of implementation of control measures. The WHO strongly recommends separating the analysis of potential pandemic strains from normal routine influenza diagnosis.

National and international reporting systems should take into account the new International Health Regulations (IHR 2005).

During the interpandemic period and the pandemic alert period (phase 1–5), surveillance in all countries should target the rapid identification of the circulating strain and the early detection and reporting of the potential pandemic strain in animals and humans. Countries affected by a pandemic threat should also determine how widespread the outbreak is, as well as whether or how efficiently human-to-human transmission is occurring. Activities during these periods should include: laboratory surveillance; a clinical case reporting system including reporting from hospitals; an early warning system for investigating clusters of acute respiratory disease; a basic system for animal surveillance; and collaboration with a reference laboratory to identify non-typable influenza. Activities in countries affected by animal outbreaks should also include case investigation and contact tracing, cluster investigation and health monitoring of high-risk groups. Desirable surveillance activities during the pre-pandemic phase may include pneumonia surveillance and monitoring of antiviral drug resistance (WHO 2004).

Sentinel hospital-based surveillance is crucial for the timely triggering of public health measures and laboratory investigations. A national network of hospital sentinel surveillance should detect individuals with acute respiratory illness among hospitalised patients, unexplained deaths caused by acute respiratory illness, or clusters of severe acute respiratory illness in the community. Healthcare staff from sentinel hospitals should receive specific training for responding during influenza pandemics. Education and training needs for healthcare workers, laboratory personnel, volunteers, and others who may be working outside their area of competence and training, must be considered.

Implementation of Laboratory Diagnostic Services

As outlined by the WHO (WHO 2005e), basic diagnostic capacity must be available for the rapid confirmation of suspected human infections with a new influenza virus strain. In countries with limited resources, a network of laboratories that have their own expertise (i.e. in influenza diagnostic tests) should be established. In the interpandemic phase, all countries should have access to at least one laboratory able to offer routine influenza diagnosis, typing and subtyping, but not necessarily strain

identification. These laboratories should be made known to the WHO. The minimal laboratory capacity for these laboratories include immunofluorescence (IF) and reverse transcriptase polymerase chain reaction (RT-PCR). In the absence of laboratories able to offer routine influenza diagnosis, typing and subtyping, countries may use commercial rapid antigen detection kits. However, governments should designate resources or seek them in other countries in order to build the necessary laboratories for epidemiological surveillance.

Under optimal conditions, a national inventory of laboratories with biosafety security levels (BSL) 3 and 4 should be available. However, usually developing countries have no BSL-4 and have very few or no BSL-3 laboratories. Therefore, the available BSL-3 laboratories should be adapted to work locally (this way the diagnosis would be faster), or arrangements with BSL-3 and BSL-4 laboratories in other countries may be facilitated by the WHO. In the early stages of a pandemic, increased testing will be required when the diagnosis of pandemic strain influenza in patients with influenza-like illness cannot be assumed. Once the pandemic is established, testing of all cases will not be possible. Laboratories should provide regularly updated advice to healthcare workers. For countries whose pandemic preparedness plan includes the use of antiviral drugs, laboratory facilities will need to be in place for monitoring antiviral drug resistance. Daily reporting of cases to national authorities and the WHO, including information on the possible source of infection, must be performed (WHO 2005e).

Vaccines

Antiviral therapy and vaccination are the only options for controlling an influenza virus infection (Yen 2005, Korsman 2006). Vaccination represents the best protection against influenza (van Dalen 2005), but an appropriate vaccine cannot be developed before a new virus strain emerges. Normally, it takes at least six months to develop a vaccine and manufacture it on a large scale (Flemming 2005). But even then, most countries without production facilities will have no access to vaccines during the first pandemic wave, as a result of limited global production capacity and concentration of these facilities in developed countries.

Countries with production facilities should support and ensure by all means that rapid and large-scale production can take place during a pandemic. In some developed nations, the government considers it to be its responsibility to provide the highest possible protection at the onset of a pandemic. For example, the Dutch government is currently negotiating with a manufacturer to ensure that a vaccine against any future pandemic influenza strain is available in the Netherlands as soon as possible following its development (van Dalen 2005). Meanwhile, countries without vaccine production facilities should prepare for a vaccination programme to be implemented as soon as vaccines against the pandemic become available (WHO 2005e).

Plans for pandemic vaccine use should include: designation of mass immunisation clinics, strategies for staffing and staff training, strategies to limit distribution to persons in the priority groups, vaccine storage capacity of the cold chain, identification of current and potential contingency depots, vaccine security (theft prevention) during its transport, storage and use in clinics. Some examples of priority groups are animal or bird cullers, veterinarians and farmers in the case of animal or

avian influenza; healthcare workers and workers in essential services when a pandemic is imminent or established (WHO 2005e).

Antiviral Drugs

Antiviral drugs include M2 inhibitors, which are ion channel blockers (amantadine and rimantadine), and the neuraminidase inhibitors (oseltamivir and zanamivir) (Hoffmann 2006b). The emergence of resistant variants is a concern with the use of any antiviral drugs. Treatment with M2 inhibitors can cause emergence of fully pathogenic and transmissible resistant variants in at least 30 % of individuals (Hayden 1997). Moreover, M2 inhibitors are ineffective against H5N1 *in vitro* (Lipatov 2004).

After treatment with neuraminidase inhibitors, resistant variants were initially found in approximately 4 % to 8 % of children and < 1 % of adults (McKimm-Breschkin 2003, Stilianakis 2002), and were identified later in 18 % of Japanese children during treatment with oseltamivir (Kiso 2004). The emergence of resistant influenza A (H5N1) variants during oseltamivir treatment was recently reported in two Vietnamese patients (de Jong 2005). Influenza A (H5N1) viruses with a H274Y substitution in the neuraminidase gene, which confers high-level resistance to oseltamivir (Gubareva 2001), were isolated from both patients. Even though oseltamivir was administered at the recommended dose and duration (75 mg twice daily for five days, with a weight-based reduction in the dose for children less than 13 years old) and treatment was started when the greatest clinical benefit could be expected (within 48 hours after the onset of symptoms), both patients died. These observations suggest that the development of drug resistance contributed to the failure of therapy in these patients. The authors conclude that strategies aimed at improving antiviral efficacy (e.g., the use of higher doses, longer durations of therapy, or combination therapy) deserve further evaluation.

New routes of administration of antivirals should also be explored, as altered pharmacokinetics in severely ill influenza patients, who may be affected by diarrhoea, have been reported (Hien 2004).

There are concerns that young children and patients with intellectual or coordination impairments are not able to inhale zanamivir properly (Imuta 2003). However, as resistance against oseltamivir can emerge during the currently recommended regimen, and as zanamivir might be less prone to the development of resistance mutations (Moscona 2005), zanamivir might be included in the treatment arsenal for influenza A (H5N1) virus infections.

Drug Stockpiling

Some governments have recently opted for stockpiling of oseltamivir. The number of courses of oseltamivir to be stockpiled by each country depends on existing resources and population size. The World Health Organisation has been urging countries to stockpile the drug in advance (Abbott 2005). For example, the Dutch government has stockpiled approximately 225,000 courses of oseltamivir (Groeneveld 2005). However, many developing countries may not be able to afford to stockpile antiviral drugs.

The cost benefit of stockpiling and the optimal strategy for antiviral use were recently investigated for the Israeli population by using data (numbers of illness episodes, physician visits, hospitalisations, and deaths) derived from previous influ-

enza pandemics. Costs to the healthcare system and overall costs to the economy, the latter including the value of lost workdays but not the potential value of lost lives, were calculated (Balicer 2005). Three strategies for the use of oseltamivir during a pandemic were defined: therapeutic use, long-term pre-exposure prophylaxis, and short-term postexposure prophylaxis for close contacts of influenza patients (with index patients under treatment). The first two strategies could target either the entire population or only those at high risk of complications. The economic outcomes of each of the five strategies were compared with nonintervention. Stockpiling costs were estimated and cost-benefit ratios were calculated. The most favourable cost-benefit ratio was found when stockpiled antiviral drugs were administered either solely as a therapeutic measure or as a short-term prophylaxis for exposed contacts, a strategy termed “targeted prophylaxis” (Longini 2004). The objective of targeted strategies is to minimise drug usage while maximising effect. Therefore, in developing countries targeted prophylaxis is particularly important for saving resources.

While in most developing countries the use of antiviral agents is not expected, in developed nations the use of antiviral agents depends on whether the drugs are in short or large supply (see Table 3).

Table 3. Recommended use of antiviral agents by the Dutch Ministry of Health (adapted from Groeneveld 2005)

1. When the pandemic first reaches the Netherlands		
	Treat Index patients ^a	Provide prophylaxis to Families, housemates and other contacts of index patients: post-exposure prophylaxis
2. In a manifest pandemic or in the event of large-scale virus introduction from abroad		
If neuraminidase inhibitors are in short supply	Treat Risk groups ^b , professionals ^c , and (when relevant) people in pandemic-specific risk group ^a ; otherwise healthy people: in the event of hospitalisation due to complications	
If neuraminidase inhibitors are not in short supply	Treat Patients displaying symptoms consistent with influenza	Provide prophylaxis to Individual patients ^d and risk groups, professionals, and (where relevant) people in pandemic-specific risk group ^e

- a. As soon as possible following the appearance of the first symptoms; if treatment is not started within 48 hours, it may not be effective.
- b. Patients with serious respiratory, pulmonary or cardiovascular abnormalities or dysfunction, who, if infected with the pandemic influenza virus, would be at serious risk of pulmonary or cardiovascular function decompensation, patients with an insulin-dependent form of diabetes.
- c. All persons responsible for the diagnosis, treatment and care of influenza patients, or for logistic management of the necessary resources.
- d. Where considered appropriate by the doctor in charge of the individual patient.
- e. Following vaccination and while the virus is circulating.

Personal stockpiling of oseltamivir is strongly discouraged (Brett 2005, Moscona 2005) as this would likely lead to the use of insufficient doses or inadequate courses of therapy, and thus facilitate the emergence of oseltamivir-resistant variants. Moreover, personal stockpiling of oseltamivir further depletes the current supply that is already inadequate to meet the demand.

Antibiotics should be stockpiled for the treatment of *Staphylococcus aureus* and other secondary infections by each hospital.

General Measures

Non-medical interventions have been shown to be relevant for controlling emergent infectious diseases. In Thailand, **community participation** at different levels was considered in the national program against H5N1 avian influenza. Public health workers, veterinary health workers, village health volunteers, and others participated in an ongoing national surveillance campaign beginning in October 2004 with written guidance from national authorities (Barnett 2005). The fact that 17 patients were infected with H5N1 during 2004, while only 5 were infected during 2005 in Thailand, might be reflecting an initial success in this nation's national program against H5N1 avian influenza (WHO 2005c). Intersectoral co-ordination involving non-health sectors (especially agriculture, economic, social and internal affairs) is needed. Professional networks outside the health sector (i.e. law, education, tourism) should also be engaged in the planning process.

Effective pre-event **risk communication** can reduce event-phase risk communication barriers (USDHHS 2005). Pre-event risk communication to at-risk populations and to the general population is of outstanding importance for easing social tension. By means of mass communication media (TV, radio), the general population should obtain essential information about relevant measures of hygiene, preventive measures, non-recommended actions, risk practices and other relevant issues. Mass communication media should contribute to the general knowledge of the influenza pandemic threat to create social awareness.

Training activities for healthcare professionals directed specifically at pandemic preparedness are useful in increasing healthcare workers' compliance with personal protective equipment and infection control procedures.

Finally, **pandemic simulation** exercises are useful for learning what to do in case a pandemic occurs. Around 1,000 health workers and civilians took part in an emergency drill in the Vietnamese capital Hanoi to practise the official response to a bird flu pandemic there. The rehearsal, organised by the city's authorities, involved people from the local quarter, hospitals, security and army units from Hanoi's Long Bien district where the event took place (Thanhnien 2005).

Seasonal Influenza Vaccination

Routine influenza vaccine should be administered to risk groups to decrease the chances of dual infection with the seasonal circulating influenza strain and the potential pandemic strain, facilitating reassortment. Vaccination with inactivated influenza vaccine is recommended for the following persons who are at increased risk of complications from influenza (ACIP 2005):

- persons aged ≥ 65 years;
- residents of nursing homes and other chronic-care facilities that house persons of any age who have chronic medical conditions;
- adults and children who have chronic disorders of the pulmonary or cardiovascular system, including asthma (hypertension is not considered a high-risk condition);
- adults and children who required regular medical follow-up or hospitalisation during the preceding year because of chronic metabolic diseases (including diabetes mellitus), renal dysfunction, haemoglobinopathies, or immunosuppression (including immunosuppression caused by medications or by human immunodeficiency virus [HIV]);
- adults and children who have any condition (e.g., cognitive dysfunction, spinal cord injuries, seizure disorders, or other neuromuscular disorders) that can compromise respiratory function or the handling of respiratory secretions or that can increase the risk of aspiration;
- children and adolescents (aged 6 months – 18 years) who are receiving long-term aspirin therapy and, therefore, might be at risk of developing Reye's syndrome after influenza infection;
- women who will be pregnant during the influenza season; and
- children aged 6-23 months.

Political Commitment

One of the most significant factors is political and social willingness to acknowledge and report disease dissemination. Without this key factor, no further national action to prevent pandemics can take place. High-level political support and commitment are necessary to develop a preparedness plan. Increased regional collaboration and networking may not only lead to the mutual support of people involved in the planning, but can also be used as an instrument for increasing international pressure and thus political commitment (WHO 2004). Records of past pandemics, especially that of 1918, show that a pandemic event may have disastrous consequences for any country due to its impact on the national socio-economic and political structures (PPHSN 2004).

Legal and Ethical Issues

Appropriate legislation must be in place before the pandemic event arrives. In a national disaster situation such as that of a pandemic, there are public health measures that need the support of the national legal system to be efficiently implemented. For example, a quarantine act usually authorises designated services and persons to take necessary measures to eradicate or control the spread of infectious

disease (PPHSN 2004). Similar coercive measures might be needed if vaccination became necessary to contain the pandemic.

Funding

Resource-limited countries need to formulate a feasible national influenza pandemic preparedness plan based on existing resources and the size and structure of the population. High political support is paramount for allocation of funding designated for emergency situations such as an influenza pandemic. The planning process should include identification of possible resources to fund pandemic response.

Global Strategy for the Progressive Control of Highly Pathogenic Avian Influenza

The likely progressive spread of highly pathogenic avian influenza (HPAI) into new regions will require pro-active intervention by the countries at risk, especially those situated along wild bird migration routes. Increased surveillance, detection capabilities and emergency preparedness will be required. Public awareness, along with education and training of veterinary professionals and para-professionals, farmers, marketers, poultry transport contractors and egg collectors, will be required to ensure that the disease is either prevented or detected and controlled, in order to prevent its establishment and maintenance in newly colonised ecosystems (FAO 2005).

The FAO and OIE, in collaboration with the WHO, have taken the initiative to start the process of developing the Global Strategy of Progressive Control and Eradication of HPAI. The overall goal of the strategy is to eventually eliminate HPAI from the domestic poultry sector in Asia and Europe, and prevent further introduction of HPAI into noninfected countries, thereby minimising the global threat of a human pandemic, promoting viable poultry production, enhancing robust regional and international trade in poultry and poultry products, increasing safety of food and feeds, and improving the livelihoods of all poultry sector stakeholders, especially the rural poor (FAO, OIE, WHO 2005).

Multiple opportunities exist for controlling highly pathogenic avian influenza: 1) prevent contact between wild and domestic poultry by use of screened poultry houses and treated water; 2) prevent contact between domestic waterfowl and gallinaceous poultry by use of screened houses and treated water and by exclusion of waterfowl from “wet markets”; 3) eradicate H5/H7 influenza viruses from gallinaceous poultry by culling or by using vaccines to prevent disease and transmission; 4) prevent or minimise contact between poultry, pigs, and humans and make vaccines and antiviral drugs available (Webster 2006).

Pandemic Period

During a pandemic phase the primary objective should be containment. It has been said that success depends on early identification of the first cluster of cases caused by the pandemic strain (Ferguson 2004), and on detection of a high proportion of ongoing cases (Ferguson 2005). Therefore, optimal surveillance at this point is essential for successful containment.

Surveillance

Pandemic surveillance should include monitoring of the following events: hospital admissions of suspected or confirmed cases of pandemic strain influenza, deaths among suspected or confirmed cases of influenza due to the pandemic strain, workforce absenteeism in services designated as essential, vaccine usage for routine and pandemic strain influenza vaccines (if these are available), adverse vaccine events attributed to the pandemic strain vaccine (if available), data collection for later use in the calculation of effectiveness of the pandemic strain vaccine, monitoring pneumococcal vaccine use and adverse events associated with its use (if this vaccine is available and being used), and monitoring of antiviral use and adverse events that may be attributed to antiviral use, if applicable. Moreover, a mechanism for data aggregation, interpretation and transmission for decision making must be ensured. The daily reporting of cases to national authorities and to the WHO, including information on the possible source of infection, must be performed (WHO 2005e).

Treatment and Hospitalisation

While the numbers of affected persons are still small, patients with suspected or proven influenza A (H5N1) should be hospitalised in isolation for clinical monitoring, appropriate diagnostic testing, and antiviral therapy. Both the patients and their families require education in personal hygiene and infection-control measures. The management is based on supportive care with provision of supplementary oxygen and ventilatory support. Patients with suspected influenza A (H5N1) should promptly receive a neuraminidase inhibitor pending the results of diagnostic laboratory testing (WCWHO 2005). For more details, see Hoffmann 2006.

Human Resources: Healthcare Personnel

High-efficiency masks (NIOSH-certified N-95 or equivalent), long-sleeved cuffed gowns, face shield or eye goggles, and gloves are recommended for healthcare workers in contact with patients. When feasible, the number of healthcare workers with direct patient contact and the access to the environment of patients should be limited. Healthcare workers involved in high-risk procedures (e.g., aerosol-generating procedures) should be considered for pre-exposure prophylaxis (WCWHO 2005).

Geographically Targeted Prophylaxis and Social Distancing Measures

Models can be used to estimate influenza-associated morbidity and mortality. Even though current models used for developed countries are not useful for developing countries, some interesting principles may be considered for the latter.

By means of a simulation model of influenza transmission in Southeast Asia, it was recently suggested that the elimination of a nascent pandemic may be feasible using a combination of geographically targeted prophylaxis and social distancing measures, if the basic reproduction number of the new virus is below 1.8 (Ferguson 2005). The basic reproduction number R_0 (Anderson 1992) quantifies the transmis-

sibility of any pathogen, and is defined as the average number of secondary cases generated by a typical primary case in an entirely susceptible population. A disease can spread if $R_0 > 1$, but if $R_0 < 1$, chains of transmission will inevitably die out. Hence, the goal of control policies is to reduce R_0 to levels below 1. However, from this simulation model, Ferguson concluded that a number of key criteria must be met for a high probability of success: (1) rapid identification of the original case cluster, (2) rapid, sensitive case detection and delivery of treatment to targeted groups, (3) effective delivery of treatment to a high proportion of the targeted population, (4) sufficient stockpiles of drug, (5) population co-operation with the containment strategy and, in particular, any social distance measures introduced, (6) international co-operation in policy development, epidemic surveillance and control strategy implementation. Successful containment is unlikely if R_0 exceeds 1.8 for the new pandemic strain.

In a stochastic influenza simulation model using a similar approach (Longini 2005), it was suggested that combinations of targeted antiviral prophylaxis, pre-vaccination, and quarantine could contain strains with an R_0 as high as 2.4. In fact, the World Health Organisation welcomed both the pandemic influenza response modelling papers aforementioned (WHO 2005g). However, there are critical arguments with respect to the simulation models. For example, it has been noticed that Longini's article assumed that oseltamivir would be useful in a pandemic, but oseltamivir may not be effective on all new avian flu viruses (Chung 2005). Moreover, oseltamivir was ineffective in 50 % of patients in Thailand (Fergusson 2005). Handling the ever-changing disease pattern of pandemic avian influenza requires a contingency plan to prepare for the worst scenario. Such a worst-case scenario model provides valuable information for resource planning, for example, the number of ventilators, the amount of intensive care, and even funeral facilities that will be required (Chung 2005).

Measures to increase the social distance have been used in past pandemics and remain important options for responding to future pandemics (WHO 2005f). These measures include travel or movement restrictions (leaving and entering areas where infection is established), closure of educational institutions, prohibition of mass gatherings, isolation of infected persons and those suspected of being infected, and quarantine of exposed individuals or travellers from areas where pandemic strain influenza infection is established (WHO 2005e). However, the effectiveness of some distancing measures that were successfully implemented for the contention of SARS remains to be demonstrated for influenza. The reason for this is that SARS patients are not infectious prior to the onset of illness, whereas influenza patients are infectious before they develop apparent symptoms (Ho 2004).

Tracing of Symptomatic Cases

Influenza is predicted to be very difficult to control using contact tracing because of the high level of presymptomatic transmission. In addition, contact tracing for influenza would probably be unfeasible because of the very short incubation (2 days) and infectious (3–4 days) periods of that disease (Fraser 2004).

Border Control

During the SARS outbreak, body temperature screening was commonly performed on air passengers. This way, individuals with fever were prohibited from boarding

aeroplanes. A hospital near each airport was designated to house, diagnose, and treat any passengers found with fever at the airport (Ho 2004). However, with an infrared body temperature screening device, only patients with symptomatic influenza disease would be detected.

Hygiene and Disinfection

Recommendations for “respiratory hygiene” such as covering one's mouth when coughing and avoiding spitting, have been made more on the basis of plausible effectiveness than controlled studies (CDC 2003). The influenza virus can remain viable on environmental surfaces and is believed to be transmissible by hands or fomites (WHO 2006). Most, but not all, controlled studies show a protective effect of hand washing in reducing upper respiratory tract infections; most of the infections studied were likely viral, but only a small percentage were due to influenza (Fasley 1999). No studies appear to address influenza specifically (WHO 2006).

Risk Communication

A risk communication strategy, flexible enough to increase its intensity during different pandemic phases, should be established. The most appropriate and effective media that can be employed should be identified. It is advisable to identify an official spokesperson during the interpandemic phase who will continue to carry out that task during subsequent phases of the pandemic. Information sources should be credible and acceptable to the public, e.g. WHO, CDC, FAO. The spokesperson(s) would ideally be someone associated with authority. Generation of fear and panic should be avoided, while practical information should remain accessible to everyone (PPHSN 2004).

Conclusions

A major influenza pandemic will have devastating consequences, with uncalculable risks for human health, global economy and political and social stability in most countries. Robust financial resources and a good medical infrastructure may help alleviate some of these consequences; however, developing countries are likely to be faced with insufficient or non-existent stocks of antiviral drugs, and without an appropriate vaccine.

The pandemic risk in developing countries is closely related to human exposure. In some African, Latin American and Southeast Asian countries, people sleep in the same places as poultry. In Southeast Asia and beyond, markets with live poultry pose a risk of human transmission (Webster 2004). Reducing human exposure requires education about handling poultry and a fundamental change in cultural attitudes towards human-animal interactions in many parts of the world (World Report 2005). Simple precautionary measures for food preparation, poultry handling, and avoidance of contaminated water are essential until effective human vaccines for H5N1 viruses become available (Hayden 2005). Therefore, pandemic preparedness in developing countries should consider funds for public education to generate cultural changes and improvements in hygiene.

Five essential action strategies to reduce the risk of a pandemic outlined by the WHO are:

- Reduction of human exposure
- Intensifying capacity for rapid containment (stockpiling of enough cycles of antiviral drugs for targeted prophylaxis combined with social distance measures)
- Strengthening early warning systems
- Rapid investigation of cases and clusters
- Building general capacity for healthcare.

If transmission of a new pandemic strain begins in human beings, the speed at which influenza spreads will depend on how early it is detected, and how fast the international community can mobilise and deliver assistance, including providing antiviral drugs for prophylactic use. Therefore, in addition to a national preparedness plan, governments should actively seek international collaborations with neighbouring countries (Ho 2004). “Without international co-operation, no nation can consider itself safe”, warned WHO Director-General Lee Jong-Wook.

In a meeting convened by the WHO in Geneva in November 2005, representatives of several low-income countries expressed concerns about the lack of action to promote equitable distribution of drug stockpiles and vaccines in the event of a pandemic. Many countries are too poor to buy drug stockpiles and have no capacity for manufacturing vaccine or generic versions of drugs (World Report 2005). Western nations are stockpiling antiviral drugs and developing vaccines, leaving poor and middle-income countries to worry that they will not have access to these potential lifesavers. At this meeting, none of the proposals directly addressed the question of equitable access to medicines and vaccines should a pandemic occur (Enserink 2005).

Support of developing countries from Western nations should precede the pandemic. Once the pandemic starts, it will be too late. Pandemics do not have frontiers, so international co-operation and equitable distribution of resources should start as soon as possible.

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124 Pandemic Preparedness

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126 Pandemic Preparedness

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Chapter 6: Vaccines

Stephen Korsman

Introduction

Vaccines are apathogenic entities that cause the immune system to respond in such a way, that when it encounters the specific pathogen represented by the vaccine, it is able to recognise it – and mount a protective immune response, even though the body may not have encountered that particular pathogen before.

Influenza viruses have been with mankind for at least 300 years, causing epidemics every few years and pandemics every few decades. They result in 250,000 – 500,000 deaths, and about 3-5 million cases of severe illness each year worldwide, with 5-15 % of the total population becoming infected (WHO 2003). Today, we have the capability to produce 300 million doses of trivalent vaccine per year – enough for current epidemics in the Western world, but insufficient for coping with a pandemic (Fedson 2005).

The influenza vaccine is effective in preventing disease and death, especially in high risk groups, and in the context of routine vaccination, the World Health Organization reports that the “influenza vaccine is the most effective preventive measure available” (WHO 2005e). With regard to the present fear of an imminent influenza pandemic, “Vaccination and the use of antiviral drugs are two of the most important response measures for reducing morbidity and mortality during a pandemic.” (WHO 2005d).

Vaccine Development

History

The concept of vaccination was practiced in ancient China, where pus from smallpox patients was inoculated onto healthy people in order to prevent naturally acquired smallpox. This concept was introduced into Europe in the early 18th century, and in 1796, Edward Jenner did his first human experiments using cowpox to vaccinate (vacca is Latin for cow) against smallpox. In 1931, viral growth in embryonated hens’ eggs was discovered, and in the 1940s, the US military developed the first approved inactivated vaccines for influenza, which were used in the Second World War (Baker 2002, Hilleman 2000). Greater advances were made in vaccinology and immunology, and vaccines became safer and mass-produced. Today, thanks to the advances of molecular technology, we are on the verge of making influenza vaccines through the genetic manipulation of influenza genes (Couch 1997, Hilleman 2002).

Yearly Vaccine Production

All vaccines in general use today are derived from viruses grown in hens’ eggs, and contain 15 µg of antigen from each of the three strains selected for that year’s vaccine – two influenza A strains (H1N1 and H3N2) and one influenza B strain. From

the selection of the strains to be used in the vaccine, all the way to the final vaccine, is a lengthy process that may take up to 6–8 months.

Selection of the yearly vaccine strain

Throughout the year, 110 national influenza surveillance centres and 4 WHO collaboration centres in 82 countries around the world watch the trends in circulating strains of influenza. Genetic data is collected, and mutations identified. The WHO identifies the strains that are likely to most resemble the strains in circulation during the next year's winter seasons, and this information is shared with vaccine producers, who begin preparation for vaccine production.

This decision is made each year in February for the following northern hemisphere winter and September for the following southern hemisphere winter. Details of the planned February 2006 meeting can be seen on the WHO website (WHO 2005k).

For the northern hemisphere winter season from the end of 2004 to the beginning of 2005, the recommendations were as follows (WHO 2005h-i):

- an A/New Caledonia/20/99(H1N1)-like virus
- an A/Fujian/411/2002(H3N2)-like virus
- a B/Shanghai/361/2002-like virus

For the southern hemisphere winter season of mid-2005, the recommendations were:

- an A/New Caledonia/20/99(H1N1)-like virus
- an A/Wellington/1/2004(H3N2)-like virus
- a B/Shanghai/361/2002-like virus

For the northern hemisphere winter season of 2005-2006, the recommendations are:

- an A/New Caledonia/20/99(H1N1)-like virus
- an A/California/7/2004(H3N2)-like virus
- a B/Shanghai/361/2002-like virus

For the southern hemisphere winter season of mid-2005, the recommendations are:

- an A/New Caledonia/20/99(H1N1)-like virus
- an A/California/7/2004(H3N2)-like virus
- a B/Malaysia/2506/2004-like virus

For example, A/New Caledonia/20/99(H1N1) means that it is an influenza A, type H1N1, the 20th isolate from New Caledonia in 1999. One can see that the H1N1 influenza A in the vaccine still represents the circulating strain, while the H3N2 virus has changed over time. Obviously, A/Fujian/411/2002 was not a good prediction in 2004. As a matter of fact, the rate of vaccine failure was unusually high during the winter season 2004/2005.

Processes involved in vaccine manufacture

Shortly after the WHO announces the anticipated circulating strains for the coming season, vaccine manufacturers start making the new vaccine strain. If the strain

chosen to be represented in the vaccine is the same as that used in the previous vaccine, the process is faster.

First, the CDC, or other reference source, take the strains to be used and grow them in combination with a strain called PR8 (H1N1 A/PR/8/34) which is attenuated so that it is apathogenic and unable to replicate in humans (Beare 1975, Neumann 2005). This allows reassortment to occur, resulting in a virus containing six PR8 genes along with the haemagglutinin (HA) and neuraminidase (NA) of the seasonal strain. This new virus is then incubated in embryonated hens' eggs for 2-3 days, after which the allantoic fluid is harvested, and the virus particles are centrifuged in a solution of increasing density to concentrate and purify them at a specific density. Then, the viruses are inactivated using formaldehyde or β -propiolactone, disrupted with detergent, and the HA and NA are purified. Finally, the concentrations are standardized by the amount of hemagglutination that occurs (Hilleman 2002, Potter 2004, Treanor 2004).

In about June/July, the strains are tested to ensure adequate yield, purity, and potency. After this, the three strains – two influenza A strains and one influenza B strain, which were all produced separately – are combined into one vaccine, their content verified, and packaged into syringes for distribution.

Production capacity

At present, the world has a production capacity of about 300 million trivalent influenza vaccines per year, most of which is produced in nine countries – Australia, Canada, France, Germany, Italy, Japan, the Netherlands, the United Kingdom, and the United States. In 2003, only 79 million doses were used outside of these countries and Western Europe. A further 13.8 million vaccines were produced and used locally in Hungary, Romania, and Russia (Fedson 2005).

Approximately 4-5 million doses of the live attenuated virus vaccine are produced per year.

Types of Influenza Vaccine

The different types of vaccines in use today for influenza can be divided into killed virus vaccines and live virus vaccines. Other vaccines of these two types are under development, as well as some that do not fall into either category, where a degree of genetic manipulation is involved.

Killed vaccines

Killed virus vaccines can be divided into whole virus vaccines, and split or subunit vaccines.

Whole virus vaccines were the first to be developed. The influenza virus was grown in the allantoic sac of embryonated hens' eggs, subsequently purified and concentrated using red blood cells, and finally, inactivated using formaldehyde or β -propiolactone. Later, this method of purification and concentration was replaced with centrifuge purification, and then by density gradient centrifugation, where virus particles of a specific density precipitate at a certain level in a solution of increasing density. Subsequently, filter-membrane purification was added to the methods available for purification/concentration (Hilleman 2002, Potter 2004).

Whole virus vaccines are safe and well tolerated, with an efficacy of 60-90 % in children and adults.

Split vaccines are produced in the same way as whole virus vaccines, but virus particles are disrupted using detergents, or, in the past, ether.

Subunit vaccines consist of purified HA and NA proteins, with the other viral components removed. Split and subunit vaccines cause fewer local reactions than whole virus vaccines, and a single dose produces adequate antibody levels in a population exposed to similar viruses (Couch 1997, Hilleman 2002, Potter 2004). However, this might not be sufficient if a novel pandemic influenza virus emerges, and it is believed that two doses will be required.

Inactivated influenza virus vaccines are generally administered intramuscularly, although intradermal (Belshe 2004, Cooper 2004, Kenney 2004) and intranasal (mucosal) routes (Langley 2005) are being investigated.

Live vaccines

Cold-adapted live attenuated influenza virus (CAIV) vaccines, for intranasal administration, have been available in the USA since July 2003, and in the former Soviet Union, live attenuated influenza vaccines have been in use for several years. The vaccine consists of a master attenuated virus into which the HA and NA genes have been inserted. The master viruses used are A/Ann Arbor/6/60 (H2N2) and B/Ann Arbor/1/66 (Hoffman 2005, Palese 1997, Potter 2004). The vaccine master virus is cold-adapted – in other words, it has been adapted to grow ideally at 25 degrees Celsius, which means that at normal human body temperature, it is attenuated. The adaptation process has been shown to have caused stable mutations in the three polymerase genes of the virus, namely PA, PB1, and PB2 (Hilleman 2002, Potter 2004).

The advantages of a live virus vaccine applied to the nasal mucosa are the development of local neutralising immunity, the development of a cell-mediated immune response, and a cross-reactive and longer lasting immune response (Couch 1997).

Of concern in the CAIV vaccine, is the use in immunocompromised patients (safety ?) and the possible interference between viral strains present in the vaccine which might result in decreased effectiveness. Damage to mucosal surfaces, while far less than with wild-type virulent influenza viruses, may lead to susceptibility to secondary infections. Safety issues, however, do not seem to be a problem in immunocompetent individuals. Of greater concern for the future is the possibility of genetic reversion – where the mutations causing attenuation change back to their wild-type state – and reassortment with wild-type influenza viruses, resulting in a new strain. However, studies done to test for this have not detected problems so far (Youngner 1994).

Vaccines and technology in development

It is hoped that **cell culture**, using Madin-Darby Canine Kidney (MDCK) or Vero (African green monkey kidney) cells approved for human vaccine production, may eventually replace the use of hens' eggs, resulting in a greater production capacity, and a less labor-intensive culturing process. However, setting up such a facility takes time and is costly, and most vaccine producers are only now beginning this process.

Reverse genetics allows for specific manipulation of the influenza genome, exchanging genome segments for those desired (Palase 1997, Palese 2002b). Based on this method, several plasmid-based methods (Neumann 2005) for constructing new viruses for vaccines have been developed, but are not yet in use commercially. A number of plasmids, small circular pieces of DNA, containing the genes and promoter regions of the influenza virus, are transfected into cells, which are then capable of producing the viral genome segments and proteins to form a new viral particle. If this method could be used on a larger scale, it may simplify and speed up the development of new vaccines – instead of the cumbersome task, for the live attenuated vaccines, of allowing reassortment in eggs, and then searching for the correct reassortment (6 genes from the vaccine master strain, and HA and NA from the selected strain for the new vaccine), the vaccine producers could simply insert the HA and NA genes into a plasmid.

DNA vaccines have been tested for a variety of viral and bacterial pathogens. The principle upon which the vaccine works is inoculation with DNA, which is taken up by antigen presenting cells, allowing them to produce viral proteins in their cytosol. These are then detected by the immune system, resulting in both a humoral and cellular immune response (Hilleman 2002).

Vaccines to conserved proteins have been considered, and among the candidates are the M2 and the NP proteins. It is hoped that, by producing immunity to conserved proteins, i.e. proteins that do not undergo antigenic change like HA and NA do, a vaccine can be produced that does not need to be “reinvented” each year. This is also on the WHO’s agenda for a pandemic vaccine (Couch 2005). Such vaccines have been shown to be effective in laboratory animals, but data are not available for human studies. “Generic” HA-based vaccines, aimed at conserved areas in the protein, are also being considered (Palese 2002b).

Adjuvants have been used in a number of vaccines against other pathogens, and are being investigated for a role in influenza vaccines. The purpose of adjuvants is to increase the immune response to the vaccine, thus allowing either a decrease in antigen dose, a greater efficacy, or both. Alum is the only adjuvant registered in the United States, and MF59, an oil/water emulsion, has been used in influenza vaccines in Europe since 1997 (Wadman 2005). A vaccine using the outer membrane proteins of *Neisseria meningitidis* as an adjuvant has shown success in early clinical trials (Langley 2005).

Attenuation by deletion of the gene NS1 or decreasing the activity of NS1 is being investigated. NS1 produces a protein that inhibits the function of interferon alpha (IFN α). If a wild-type influenza virus infects a person, the NS1 protein antagonises IFN α , which has an antiviral effect. An infection with a NS1-deficient virus would quickly be overcome by the immune system, hopefully resulting in an immune response, but with no symptoms (Palese 2002b).

Replication-defective influenza viruses can be made by deleting the M2 or the NS2 genes (Hilleman 2002, Palese 2002b). Only a single round of replication can occur, with termination before the formation of infectious viral particles. Protein expression will result in an immune response, and there is no danger of infection spreading to other cells or people.

Efficacy and Effectiveness

Antibody response, determined by measuring haemagglutination inhibition titers, is used as a serological marker of the immunological response to the vaccine, or efficacy. In persons primed by previous exposure to viruses of the same subtype, antibody response is similar for the various types of vaccines. However, in persons without such previous exposure (either through vaccination or through natural infection), response is poorer in the split and subunit vaccines, where two doses are required.

In healthy primed adults, efficacy after one dose ranges from 80-100 %, while in unprimed adults, efficacy enters into this range after two doses. In other populations, efficacy is lower:

Table 1: Efficacy of influenza vaccination*

Population	Efficacy
Healthy adults and most children	80-100 %
Renal failure (chronic)	66 %
Renal transplant	18-93 %
Hemodialysis	25-100 %
Bone marrow transplant	24-71 %
Cancer	18-60 %
HIV infection	15-80 %

*adapted from Pirofzki 1998, Potter 2004, Musana 2004

Effectiveness, usually defined by prevention of illness, is generally slightly lower, with 70-90 % effectiveness in children and healthy adults under the age of 65. In those above 65 years of age, a lower rate of 30-40 % is seen. However, the vaccine is 20-80 % effective in preventing death from influenza in persons older than 65 years, with revaccination each year reducing mortality risk more than a single vaccination (Govaert 1994, Gross 1995, Nichol 1994, Partriarca 1985, Voordouw 2004). In patients with previous myocardial infarctions (MI), a study by Gurfinkel et al. (2004) showed a reduction in the one year risk of death (6 % in the vaccinated group, 13 % in the control group) and combination of death, repeat MI, or rehospitalisation (22 % versus 37 %), possibly due to a non-specific effect of immune responsiveness. Further studies are planned to evaluate the impact of influenza vaccination on acute coronary syndromes.

Vaccination of caregivers against influenza also reduces the exposure of vulnerable populations to influenza.

Studies have been done on effectiveness in terms of health benefits and cost in several healthy populations (Bridges 2000, Langley 2004, Monto 2000, Wilde 1999). They suggest that, while individual health benefits from vaccination certainly exist, as do reductions in days absent from work, vaccinating healthy working adults may not provide cost savings when compared to loss of productivity and days taken off due to illness. Vaccinating health care professionals is recommended, not only because of health benefits and reduced days absent from work, but because it is believed that hospital employees tend to report to work in spite of having an acute febrile illness. Previous studies have shown that vaccinating health care professionals reduces nursing home and hospital-acquired influenza infections (Pachuki 1989, Potter 1997).

Side Effects

Guillain-Barré Syndrome is seen as the most dangerous side effect of influenza vaccines, aside from manifestations of egg allergy. It is, however, rare: the annual reporting rate decreased from a high of 0.17 per 100,000 vaccinees in 1993-1994 to 0.04 in 2002-2003 (Haber 2005).

The most frequent side effects are pain, redness, and swelling at the injection site (10-64 %) lasting 1-2 days, and systemic side effects such as headache, fever, malaise, and myalgia in about 5 % of vaccinees (Belshe 2005, Musana 2004, Potter 2004). These side effects are largely due to a local immune response, with interferon production leading to systemic effects. Local side effects are more common with whole virus vaccines than subunit or split vaccines, and also more common with intradermal vaccination than intramuscular vaccination.

Since the inactivated vaccines do not contain live virus, they cannot cause influenza infection – often respiratory illness is incorrectly attributed to influenza vaccination. Live attenuated virus vaccines do contain live virus; however, side effects are rare, with a runny nose, congestion, sore throat, and headache being the most commonly reported symptoms, with occasional abdominal pain, vomiting, and myalgia (Musana 2004). They are not recommended for use in children below the age of 5 years, although a study by Piedra et al. (Piedra 2005) showed safety in children above the age of 18 months. Controversies have arisen around the possibility of exacerbated asthma in children between 18-34 months of age (Bergen 2004, Black 2004, Glezen 2004). It should be noted, however, that these vaccines should be avoided in immunocompromized patients.

Recommendation for Use

Indications

Groups to target

The primary groups to be targeted for vaccination can be memorized with an easy mnemonic – FLU-A (Musana 2004).

F – facilities such as nursing homes or chronic care facilities.

L – likelihood of transmission to high risk persons – healthcare workers and care providers can transmit influenza to patients, as can other employees in institutions serving the high risk population groups, as well as people living with individuals at high risk.

U – underlying medical conditions such as diabetes mellitus, chronic heart or lung disease, pregnancy, cancer, immunodeficiency, renal disease, organ transplant recipients, and others.

A – age > 65 years, or between 6-23 months of age

Since the risk of influenza rises linearly from the age of 50 years, some promote the vaccination of those aged between 50 and 64 in addition to those above 65 years of age. In a study of health professional attitudes to such a policy in England, both sides were equally divided (Joseph 2005). Vaccination for those above 50 years of

age is recommended in the USA, while all those above 6 months are offered vaccination in Canada.

In the era of a potentially pending pandemic, other groups also have importance for targeting – poultry workers in the Far East are being vaccinated to prevent infection with circulating human influenza strains. This vaccine will not protect against avian influenza strains, but will help prevent dual infection, if infection with avian influenza does occur, thereby reducing opportunities for reassortment of two strains in one human host. For the same reason, travelers to areas where avian influenza is present are advised to be vaccinated against human influenza (Beigel 2005).

Guidelines

The World Health Organisation makes the following recommendations on who should receive influenza vaccines (WHO 2005b-c, WHO 2005f):

- Residents of institutions for elderly people and the disabled.
- Elderly, non-institutionalized individuals with chronic heart or lung diseases, metabolic or renal disease, or immunodeficiencies.
- All individuals > 6 months of age with any of the conditions listed above.
- Elderly individuals above a nationally defined age limit, irrespective of other risk factors.

Other groups defined on the basis of national data and capacities, such as contacts of high-risk people, pregnant women, healthcare workers and others with key functions in society, as well as children aged 6–23 months.

The CDC guidelines are similar, with a few additions (Harper 2004, CDC 2005) -

- Residents of nursing homes and long-term care facilities
- Persons aged 2-64 years with underlying chronic medical conditions
- All children aged 6-23 months
- Adults aged > 65 years – high risk
- Adults aged > 50 years – recommended
- All women who will be pregnant during the influenza season
- Children aged 6 months – 18 years on chronic aspirin therapy
- Healthcare workers involved in direct patient care
- Out-of-home caregivers and household contacts of children aged 0-23 months

South Africa has the following guidelines (summarised from Schoub 2005), dividing the population into 4 groups who may receive the vaccine –

- Category 1 – At risk persons (i.e. at risk for complications of influenza)
 - All persons over the age of 65 years
 - Persons with chronic pulmonary or chronic cardiac disease
 - Immunosuppressed persons
 - Pregnancy – women who will be in the second or third trimester during the winter season. Vaccination is contraindicated in the first trimester.

- Children with chronic pulmonary or cardiac diseases as well as immunosuppressed children. Children on aspirin therapy should also be immunised because of the risk of Reye's syndrome.
- Category 2 – Contacts of high-risk persons - healthcare workers, caregivers of the elderly and high-risk patients, and persons living with high risk persons.
- Category 3 – Workplace vaccination.
- Category 4 – Personal protection.

Australian guidelines (Hall 2002) -

- Everyone 65 years of age and older
- Aboriginal and Torres Strait Islander people 50 years of age and older
- People six months of age and older with chronic illnesses requiring regular medical follow-up or hospitalisation in the previous year
- People six months of age and older with chronic illnesses of the pulmonary or circulatory systems (except asthma)
- Residents of nursing homes or long-term care facilities
- Children and teenagers aged six months to 18 years on long-term aspirin therapy (because aspirin treatment puts them at risk of Reye's syndrome if they develop a fever)
- Healthcare and other workers providing care to the high-risk groups above.
- Other groups for whom influenza immunisation should be considered include pregnant women, overseas travelers and persons infected with HIV.

Most countries with guidelines will have similar recommendations. Canada, although having similar recommendations for priority groups, actively encourages vaccination of everyone above the age of 6 months (Orr 2004).

If a pandemic becomes a reality, recommendations will likely extend to everyone. However, frontline workers such as healthcare personnel, as well as police forces and military personnel, might be high priority targets.

Contraindications

Contraindications to influenza vaccination are:

- egg allergy – the vaccines are made in eggs, and, although rare, severe allergic reactions such as anaphylaxis can occur.
- acute febrile illness – vaccination should be delayed. Minor illnesses such as mild upper respiratory tract infections or allergic rhinitis are not contraindications.
- first trimester of pregnancy has in the past been seen as a contraindication. However, the ACIP recommendations changed in 2004, and currently the guidelines say that vaccination can occur in any trimester (Bettes 2005, Harper 2004).
- previous Guillain-Barré syndrome has in the past been considered as a contraindication, but this is now no longer a contraindication for the use of inactivated vaccine. (Fleming 2005).

Contraindications to vaccination with live attenuated vaccine are (Medimmune 2005):

- age < 5 or > 65 years.
- immunocompromised patients – the use of the live-attenuated vaccine is contraindicated, and inactivated vaccines should be used instead. Caution should be used when giving the vaccine to those who may come into contact with immunocompromised patients, as this caused controversy in 2004 when vaccine supplies were limited (Manion 2005). HIV-infected individuals may not have significant immune suppression in the early years of their HIV infection, and it is accepted that certain live attenuated vaccines, such as those for measles and varicella, can be used in these patients. Little information is available on the use of live attenuated influenza vaccine in HIV-infected people, but what is available suggests that this vaccine is safe in adults who are in the CDC class A1-2, and in children who are in the CDC class N1-2 or A1-2, i.e. asymptomatic or mildly symptomatic, with CD4 counts higher than 200/μl in adults (King 2000, King 2001). Both studies conclude that inadvertent vaccination or exposure to the attenuated virus is unlikely to result in significant adverse effects. However, it should be noted that small numbers of patients were involved, and until sufficient data are obtained, extreme caution should be exercised.
- previous Guillain-Barré syndrome.
- children under the age of 18 years who are receiving aspirin therapy should not receive live vaccine, as it is a risk for Reye's syndrome. They should receive inactivated vaccine instead.
- In addition,
 - safety in asthma sufferers and patients with underlying medical conditions that put them at risk for wild type influenza infections has not been established.
 - safety regarding teratogenicity and breast milk excretion has not been established in pregnant women, who should receive inactivated vaccine instead.
 - parenteral administration is contraindicated – mucosal administration via nasal spray is the correct usage.
 - administration with other vaccines should be avoided – within 4 weeks before or after a live vaccine, and within 2 weeks before or after an inactivated vaccine.

Dosage / use

Inactivated vaccine

Children

- 6-35 months – 0.25 ml in anterolateral thigh (deltoid only if adequate muscle is present)
- 3-8 years – 0.5 ml in anterolateral thigh (deltoid as above)

Adults

- 9 years onwards – 0.5 ml in deltoid muscle

Live attenuated vaccine

Children (5-8 years old)

- first vaccination – 2 doses, 60 days apart
- previous vaccination – 1 dose per season

Adults (9-49 years old)

- 1 dose per season

Companies and Products

The FDA web page on influenza vaccines can be found here:

<http://www.fda.gov/cber/flu/flu.htm>

Table 2 shows some of the available influenza vaccines, with links to FDA and package insert data.

Table 2. Influenza vaccines and manufacturers.

Manufacturer	Brand name	FDA page	Package insert
Sanofi Pasteur	Fluzone	http://www.fda.gov/cber/products/inflav e071405.htm	http://poisonevercure.150m.com/vaccines/package_inserts/AP-Fluzone_2003-04.pdf
	Fluzone – preservative free	http://www.fda.gov/cber/products/inflav e071405p2.htm	
	Inactivated Influenza Vaccine (Split Virion) BP		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=6207
	Inactivated Influenza Vaccine (Split Virion) For Pediatric Use		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=16610
	Inflexal V		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=13078
	Vaxigrip		http://www.medsafe.govt.nz/Profs/Datasheet/v/Vaxigripinj.htm
	Mutagrip		http://home.intekom.com/pharm/ranbaxy/mutagrip.html
GlaxoSmith- Kline	Fluarix	http://www.fda.gov/cber/products/inflgla083105.htm	http://emc.medicines.org.uk/emc/assets/c/html/displaydoc.asp?documentid=2038
Chiron Vaccines	Fluvirin	http://www.fda.gov/cber/products/inflchi091405.htm	http://home.intekom.com/pharm/cipla/fluvirin.html

Table 2. Influenza vaccines and manufacturers.

Manufacturer	Brand name	FDA page	Package insert
	Enzira		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=16606
Wyeth	Agrippal		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=7788
Solvay Healthcare	Influvac Sub-Unit		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=2080
	Invivac		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=15191
MASTA	MASTAFLU		http://emc.medicines.org.uk/eMC/assets/c/html/DisplayDoc.asp?DocumentID=12737
SmithKline-Beecham	X-Flu		
MedImmune Vaccines	FluMist*	http://www.fda.gov/cber/products/inflm ed081805.htm	http://poisonevercure.150m.com/vaccines/package_inserts/flumist.pdf

*FluMist is the only currently available live attenuated influenza vaccine. All others are inactivated.

Strategies for Use of a Limited Influenza Vaccine Supply

Antigen sparing methods

Several methods of reducing the amount of antigen in vaccine preparations have been investigated. Most importantly are the use of adjuvants and the exploitation of a part of the immune system designed to elicit an immune response – dendritic cells.

Adjuvants are used in a number of vaccines in current use, such as those for Diphtheria/Tetanus/Pertussis (DtaP) and *Hemophilus influenzae* (Hib). Examples of adjuvants include alum (a combination of aluminum compounds), liposomes, emulsions such as MF59, *Neisseria meningitidis* capsule proteins, immunostimulating complexes (ISCOMs), and interleukin-2. They enhance the immune response to a vaccine, allowing a lower dose to be given, while maintaining sufficient protective response (Couch 1997, Langley 2005, Potter 2004).

Dendritic cells can be exploited by giving vaccines intradermally, as they induce T cell responses, as well as T cell dependent antibody formation (La Montagne 2004, Steinman 2002). Intradermal vaccination is well established with hepatitis B and rabies vaccines, and has recently been investigated with considerable success for influenza vaccines (and in a study from 1948 (Weller 2005). 40 %, 20 %, and 10 % of the standard intramuscular dose of 15 µg antigen given intradermally produces a response similar to the full dose given intramuscularly (Belshe 2004, Cooper 2004, Kenney 2004). While the antibody titre is protective, the levels may not be as durable as those induced by intramuscular vaccination. Subjects over the age

of 60 years seem to have a weaker immune response with the intradermal vaccination, and it is likely that the intramuscular injection will be preferable in this group (Belshe 2004). Also not clear yet, is the dose-response relationship between intramuscular and intradermal routes (Kilbourne 2005). Further studies will clarify these matters. One drawback is that the local reactions can be more intense, with increased pain, swelling, and redness; however, these are still mild.

Rationing methods and controversies

In the event of a shortage of vaccine, as happened in the 2004/5 influenza season, as well as in the event of a pandemic situation, certain individuals, such as those working in the healthcare sector and in the poultry industry, and those exposed on the front lines, will need to be given priority over other groups for access to vaccines. As has happened in the past, leaders may have identify groups for urgent vaccination in order to allow for maximum functioning of essential services, while other groups may have to wait until a greater supply is available (MacReady 2005, Treanor 2004). In the event of a pandemic, this could become problematic, but recent experience in the 2004/5 shortage showed that it was managed well by most (Lee 2004), with some instances of companies buying up vaccine, leaving private practices and public health services without supply (MacReady 2005). In the UK, there have already been debates about who should get the H5N1 pandemic vaccine first – healthcare workers, or poultry workers – if H5N1 avian influenza were to reach Britain (Day 2005).

Pandemic Vaccine

The purpose of this section is not to be an exhaustive reference on avian influenza vaccine development. That is a rapidly advancing field, and the achievements of those involved will likely change the face of influenza vaccinology, and vaccinology in general. In 10 years from now, it is likely that we will look back on our current influenza vaccines and think of them as primitive. Details and advances noted now will be outdated tomorrow. This section will provide an outline of the current direction, the problems we face at the moment, and where we can hope to be in the near future.

Development

As we have seen, vaccination against influenza is a crucial weapon, not only in our fight against seasonal influenza, but against a pandemic that may come tomorrow, next year, or in the next decade. We need to prepare ourselves now.

The World Health Organisation is working with leaders of countries and vaccine manufacturers around the world to prepare for the pandemic many fear will arise out of the current H5N1 avian influenza scare (WHO 2005g).

Although it is an ongoing process, initial strains of H5 avian influenza, such as A/Duck/Singapore/97 (H5N3), have been identified for use in vaccine development (Stephenson 2005). However, it should be noted that the focus is not solely on H5 strains – H2, H6, H7, and H9 are not being ignored, although only H1, H2, H3, N1 and N2 have been found in human influenza viruses (Kilbourne 1997).

Our most urgent needs are a) a stockpile of anti-influenza drugs, b) a vaccine that matches the pandemic strain, c) expedited testing and approval of this vaccine, and

d) the capacity to mass-produce enough vaccine to provide the world with a good defense. At present, all of these are still in their infancy.

A matching vaccine will require knowledge of the pandemic strain, and until the next pandemic begins, we will not know for certain what that strain will be. Current efforts are working with a number of strains, mostly H5 strains, as this seems to be the most likely origin at the present time.

The technology to rapidly develop such a vaccine needs to be fully developed. At present, there are several methods being used to develop candidate vaccines.

- Cell culture systems, using Vero or MDCK cell lines, are in development, and will increase our production capacity. The cells could be grown on microcarriers – glass beads – to enable high volume culture (Osterholm 2005). However, these will take several years to put in place, and the cost is problematic (Fedson 2005).
- Reverse genetics is being used to design candidate vaccines – for example, H5N1 virulence genes have been removed from a laboratory strain. Attenuating the virulence of the virus is important, considering the increased mortality rate of the current highly pathogenic H5N1 avian influenza when it does enter human hosts. While the H5N1 mortality rate in humans at present doesn't necessarily reflect the mortality rate in an eventual pandemic, serious attention must be paid to the pathogenicity of the current H5N1 strain before it can be used in a vaccine.
- Plasmid systems are in development – several exist, and others are being described in the scientific literature. A generic influenza virus would supply 6 genes in plasmid form, and once the pandemic strain is identified, it would supply the HA and NA genes. DNA vaccine development experiencing a limited success.
- Apathogenic H5N3 with an adjuvant is being tested – the immune response will be against the H5 only, but the important aspect here is the use of an attenuated strain (Horimoto 2001).
- Live attenuated cold adapted virus is being considered. This may open even more doors for potential reassortment, however, and it may take considerable time to demonstrate safety in certain populations, such as the elderly and children.
- H5N2 inactivated vaccines exist for poultry, and appeared to be protective against H5N1 from 2002 and 2004, but it is expected that human vaccines will have to be better matched than poultry vaccines (Lipatov 2004).

Mock vaccines

In order to ensure that, when the time comes, a vaccine can be rapidly produced, tested, and shown to be safe, immunogenic, and protective, the WHO has asked vaccine manufacturers and scientists to start developing new vaccines based on strains that may be related to an eventual pandemic strain. These vaccines will likely never be used, and are being developed to demonstrate that when the actual pandemic vaccine is needed, the principle is sound, and the technology is in place and proven on previous vaccines – hence the term “mock vaccine”. The important

aspect is the development of established vaccines that do not need lengthy studies before they can enter the market. They need to contain viral antigens humans have not had previous exposure to, such as the H5N1 antigens, and companies need to take them through clinical trials to determine immunogenicity, dose, and safety, and ultimately be licensed for use in the same stringent procedures used for other vaccines.

Currently, an expedited system is in place for the inactivated influenza vaccines against seasonal human influenza – the whole process, from the identification of the strains to be used, to the injection in the consultation room, takes about 6-8 months, because the vaccine is an established one, and only certain aspects need to be confirmed prior to release. This same system needs to be in place for a pandemic vaccine (Fedson 2005, WHO 2004a-b).

Production capacity

In an ideal world, 12 billion doses of monovalent vaccine would be available in order to administer two doses of vaccine to every living human being.

The reality is that we do not have this much available.

Currently, the world's vaccine production capacity is for 300 million doses of trivalent vaccine per year. This amounts to 900 million doses of monovalent vaccine, if all production were shifted to make a pandemic vaccine. Considering that at least two doses will be needed, the current capacity serves to provide for only 450 million people. This is further complicated by the fact that the dose of antigen that will be required is not yet known, but studies indicate that it may be higher than current human influenza vaccines (Fedson 2005).

The world has suffered from vaccine shortages before – recently in the 2004/5 winter season, and closer to the threatening situation, in the pandemic of 1968. Furthermore, many countries do not have their own production facilities, and will rely on those countries that do. Will those countries be able to share vaccine supplies?

Transition

Osterholm asks (Osterholm 2005), “What if the pandemic were to start ...”

- tonight
- within a year
- in ten years?

The New England Journal of Medicine had an interview with Dr Osterholm, which is available online for listening to or for downloading:

<http://content.nejm.org/cgi/content/full/352/18/1839/DC1>

If the pandemic were to start now, we would have to rely on non-vaccine measures for at least the first 6 months of the pandemic, and even then, the volumes produced would not be sufficient for everyone, and some sort of rationing or triage system would be necessary. Vaccine and drug production would have to be escalated – for much later in the pandemic, as this will not make a difference in the short term. The world's healthcare system would have to plan well in order to cope with distribution when they become available – at present, it is doubted that it could handle the distribution and administration of the vaccines, never mind trying to handle that

under the pressure placed on it by a pandemic. Vaccines may only be available for the second wave of the pandemic, which tends to have a higher mortality than the initial wave.

If the pandemic starts in a year's time, it is likely that we will then have some experience in developing mock vaccines, so that a vaccine could be produced relatively quickly using a variety of the technologies currently under investigation. There would still be a significant delay, and it is likely that there would still be insufficient quantities, with rationing required.

We don't know when a pandemic will occur – but starting preparation now is essential. If the pandemic is delayed by a few years, we may well have the required vaccine production capacity to minimise the disastrous consequences.

Solutions

The WHO suggests various strategies to solve these problems (WHO 2005d) and is working with governments, scientists, vaccine and drug companies, and other role players around the world to achieve a solution.

Strategies for expediting the development of a pandemic vaccine

Shorten the time between emergence of a pandemic virus and the start of commercial production.

1. Candidate “pandemic-like” vaccines need to be made and put through trials. This will require adopting a centralized evaluation team to examine the findings of the studies and give clearance for the use of the vaccine. It would not be feasible for each medicine's evaluation team to do this for their own country. The vaccine needs to become established through “mock” trials in order to be able to be expedited in this way – then, like the current influenza vaccine, it is known, and only brief studies are required to confirm immunogenicity and safety.
2. Increased production capacity must be developed worldwide – for example, changing to cell culture vaccines. Another important means to improve production is to increase consumption – using more of the current vaccine today will not only decrease the burden of current influenza disease, as well as helping to prevent reassortment in humans infected with two strains of virus, but will ultimately enable production to be increased.

Enhance vaccine efficacy

1. Antigen sparing methods, such as intradermal injection, need to be researched more thoroughly, as they provide for a potential saving in antigen – the 1 µg of antigen (per strain) in current vaccines could be lowered considerably. If we could use one 8th of the dose, our current 900 million monovalent doses could be expanded to 7.2 billion doses – enough for 3.6 billion people, more than half of the world's population (Fedson 2005).
2. Adjuvants need to be evaluated – if immunogenicity can be enhanced, less antigen would be required for a protective immune response.

3. Mock-up vaccines must be developed and tested in clinical trials to determine the most antigen sparing formulation and the best vaccination schedule (Fedson 2005, Kilbourne 2005).
4. Newer vaccine technology needs to be developed, e.g. reverse genetics, and knowledge of epitopes in influenza to design more effective vaccines.

Controversies

A number of controversies surrounding the development of a new influenza vaccine need to be dealt with (Fedson 2005, Osterholm 2005).

Financial – patents exist for the plasmid-based methods of making virus in cell culture and the legal implications in various countries need to be examined and addressed. Will the owners of the intellectual property benefit in any way? Mock vaccines need to be made, but will probably never be sold and used. Who will fund this endeavour?

Rationing – in the event of vaccine shortage, higher risk groups will need vaccination first, along with those working on the front lines to control the pandemic. In such an event, the definition of “high risk group” may need to be revised – will it include children, for instance? Who will get the vaccine first – there is already tension over this issue in the UK: poultry farmers or healthcare workers? (Day 2005)

Equitable access will need to be ensured – countries without vaccine production, poorer countries, and developing countries will all want to have their share of the vaccine supply.

Liability issues – due to increased vaccination with current vaccines, greater attention must be paid to liability. Several countries have legislation that limits and/or covers certain liability for vaccine companies – encouraging such legislation will make vaccine companies feel more free to develop new vaccines, and increase the supply of current vaccines. When the time comes for rapid entry of pandemic vaccines into general use, such legislation will be important.

Organising

Barnett employs a Haddon Matrix to show what sort of planning needs to be done at different stages of the pandemic, from pre-pandemic to post-pandemic (Barnett 2005).

The WHO will play an important role in the process. In 2001, the Global Agenda for Influenza Surveillance and Control was established (Webby 2003, Stohr 2005). Its role is to enhance our surveillance abilities, in order to better detect a pandemic, and prepare for influenza seasons until then. It is also charged with the task of increasing our knowledge of influenza, and enhancing vaccine acceptance and use, in order to prepare us for a pandemic (WHO 2005j).

The WHO also needs to lead the address of the problems of production capacity, legislation and expedited vaccine availability, and research that needs to be done in order to reach the point where these are possible. It needs to help solve the controversies over financing, patents and intellectual property, equity for developing countries and countries not producing vaccine, and rationing of vaccine when supplies do not meet the demands of a population of more than 6 billion people.

The Ideal World – 2025

“Our goal should be to develop a new cell culture-based vaccine that includes antigens that are present in all subtypes of influenza virus, that do not change from year to year, and that can be made available to the entire world population. We need an international approach to public funding that will pay for the excess production capacity required during a pandemic.” (Osterholm 2005)

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Useful reading and listening material

Audio

- Osterholm MT. Preparing for the next pandemic. *N Engl J Med* 2005; 352: 1839-42. Audio content: <http://content.nejm.org/cgi/content/full/352/18/1839/DC1>
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Chapter 7: Laboratory Findings

Gert van Zyl

Introduction

Various diagnostic modalities have been developed since influenza virus was first characterised in 1933 (Webster 1998). These diagnostic techniques can be employed to confirm a clinical diagnosis. In this chapter the role of the most important of these tests will be discussed as well as their advantages and limitations. However the best diagnostic test has little value without appropriate good quality specimen collection and correct patient information.

Laboratory Diagnosis of Human Influenza

Appropriate specimen collection

Respiratory specimens

The timing of specimen collection is very important since the yield is the highest for respiratory specimens obtained within four days of onset of symptoms. Different types of respiratory specimens can be used. Nasal washes and nasopharyngeal aspirates tend to be more sensitive than pharyngeal swabs. In patients that are intubated, tracheal aspirates and bronchial lavages can be collected (WHO 2005a). Washes and aspirates should contain sufficient respiratory epithelium for immunofluorescence tests. Specimens without sufficient cells are however still suitable for other methods such as rapid antigen detection, virus isolation and reverse transcription-polymerase chain reaction (RT-PCR).

Swabs should be transported in virus transport medium to prevent desiccation.

All specimens should arrive at the laboratory as soon as possible to avoid any degradation. Transportation in virus transport medium on ice or with refrigeration at 2–8 degrees Celsius is recommended if any delay in transportation is expected.

Blood specimens

Blood (whole blood, serum) specimens are collected for the purpose of antibody serology (determining the presence of antibodies to influenza). Acute and convalescent serum samples 14 – 21 days apart should be collected to demonstrate a significant (at least fourfold) rise in strain-specific antibody titre.

Clinical role and value of laboratory diagnosis

Patient management

Rapid diagnosis is important if early therapeutic interventions with costly antiviral drugs are being considered – to be effective, these drugs need to be started within 48 hours after the onset of symptoms (WHO 2005a). Candidates for early treatment

are patients with underlying conditions and an increased risk of serious complications (see chapter “Clinical Presentation”). In particular, the diagnosis of influenza in elderly patients makes the clinician aware of a substantial risk of secondary bacterial infections with *Staphylococcus aureus*, *Haemophilus influenzae* and *Streptococcus pneumoniae*.

In addition, rapid testing for influenza virus plays a role in hospital infection control in reducing the spread of infection from patient to patient or from infected health care workers to high risk patients. These tests can also be used to diagnose influenza in travellers or outbreaks in semi-closed communities such as cruise ships (WHO 2005a).

Finally, diagnosis of influenza has prognostic value in healthy young adults where the disease has a short and benign course.

Surveillance

Influenza sentinel surveillance employs a variety of test and there seems a lack of standardisation even within the European region (Meerhoff 2004). Different techniques have different advantages and disadvantages. Therefore combinations of tests are used for surveillance. Rapid direct techniques such as RT-PCR (Bigl 2002) or EIA enable the fast detection of epidemics and can be used to distinguish between influenza A or B. Isolation of the virus in embryonated chicken eggs or on cell culture is necessary to subtype the viruses. Haemagglutinin and neuraminidase subtypes are respectively determined by haemagglutination inhibition assay and RT-PCR. Sequencing of PCR products is used to establish the molecular epidemiology of circulating viruses. This together with interstrain haemagglutination inhibition titres enable WHO to recommend appropriate vaccines that will most likely be protective against the circulating influenza strains. Surveillance is also important for public health policies since the health impact of a particular epidemic and cost benefit ratios of interventions such as vaccination can motivate policy makers to prioritise influenza prevention.

Laboratory Tests

Many factors should be considered in deciding which tests to use. Sensitivity, specificity, turn-around-time, repeatability, ease of performance and costs should all be taken into account. RT-PCR is generally more sensitive than serology and culture and the combination of RT-PCR with serology more sensitive than the combination of any other two methods (Zambon 2001). The sensitivity of culture is largely dependent on the laboratory where it is performed. Serology tends to be less expensive than RT-PCR but as it necessitates acute and convalescent blood specimens, diagnosis is only retrospective. Traditional culture is time-consuming but shell vial culture techniques allow diagnosis within 48-72 hours.

Direct methods

Different methods exist for direct detection of influenza viruses. Some methods such as enzyme immunoassays (EIAs) can be suitable for bedside testing, others such as direct immunofluorescence allow for the preparation of slides onsite in clinics and posting of fixed slides to a central laboratory (Allwinn 2002). RT-PCR can only be performed in well equipped laboratory facilities by trained personnel.

These methods can either detect both influenza A and B or differentiate between types (influenza A or B). The only direct technique that has the potential to differentiate between subtypes (i.e. on the basis of haemagglutinin and neuraminidase) is RT-PCR.

Immunofluorescence

For direct immunofluorescence, potentially infected respiratory epithelial cells are fixed to a slide and viral antigens contained in the cells is detected by specific antibodies which are either directly conjugated to a fluorescent dye (direct immunofluorescence) or detected by anti-antibodies linked to a fluorescent dye (indirect immunofluorescence). In both cases reactions are visualised under the fluorescence microscope and positive cells are distinguished on colour intensity and morphology of fluorescent areas. Direct immunofluorescence tends to allow faster results but is generally less sensitive than indirect immunofluorescence. Indirect immunofluorescence also has the advantage that pooled antisera can be used to screen for viral infection using a single anti-antibody conjugated to a fluorescent dye (fluorescein isothiocyanate-conjugated anti-mouse antibodies are commonly used; Stevens 1969). Immunofluorescence allows for the rapid diagnosis of respiratory specimens as long as sufficient respiratory epithelial cells are present in the specimens. However, inter-individual variation in reporting of immunofluorescence tests exists since interpretation is subjective and accuracy depends on the competence and experience of the operator.

Enzyme immuno assays or Immunochromatography assays

Enzyme immunoassays (EIAs) utilise antibodies directed against viral antigen that are conjugated to an enzyme. An incubation step with a chromogenic substrate follows and a colour change is indicative of the presence of viral antigen. Certain enzyme immunoassays as well as similar assays using immunochromatography allow for bedside testing (Allwinn 2002) taking 10-30 minutes. These rapid assays are generally more expensive than direct immunofluorescence or virus culture. Sensitivities of EIAs vary between 64% and 78% (Allwinn 2002). Different rapid tests can detect either influenza A or B virus without distinguishing the type, influenza A virus only or detect both influenza A and B and identify the type. However non of these rapid tests can differentiate between subtypes that infect humans (H1N1 and H3N2) or avian influenza subtypes (FDA, 2005). A list of rapid tests that are available can be obtained from the following link: <http://www.cdc.gov/flu/professionals/labdiagnosis.htm>.

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR is a process whereby RNA is first converted to complementary DNA (cDNA) and a section of the genome is then amplified through the use of primers that bind specifically to this target area. This allows for exponential amplification of small amounts of nucleic acid, through the action of a thermo stable DNA polymerase enzyme, which enables highly sensitive detection of minute amounts of viral genome.

Not only does RT-PCR have superior sensitivity (Steininger 2002) but it can also be used to differentiate between subtypes and conduct phylogenetic analysis (Allwinn 2002). RNA degradation of archival samples can decrease the sensitivity of RT-

PCR (Frisbie 2004). Therefore specimens should be processed as fast as possible after collection.

Isolation methods

Virus isolation or culture is a technique whereby a specimen is inoculated in a live culture system and the presence of virus infection is then detected in this culture system. Since culture amplifies the amount of virus it is more sensitive than direct methods with the exception of RT-PCR (that also employs amplification). Virus isolation is only of use if the live system or cells are sensitive for the virus that one intends to isolate.

Isolation requires the rapid transport of specimens to the laboratory since delays may lead to inactivation of virus (Allwinn 2002).

Embryonated egg culture

Specimens are inoculated into the amniotic cavity of 10-12 day embryonated chicken eggs. High yields of virus can be harvested after 3 days of incubation (WHO 2005d).

Since this technique requires the supply of fertilized chicken eggs and special incubators it is no longer used for the routine diagnosis of influenza infection. However egg isolation provides high quantities of virus and is a very sensitive culture system. Reference laboratories therefore utilise this culture system to ensure high sensitivity and to enable the production of virus stocks for epidemiological monitoring.

Cell culture

Conventional culture: Various cell-lines are utilised to isolate influenza viruses, most commonly primary monkey kidney cells and Madin-Darby canine kidney (MDCK) cells. Some authors recommend the use of trypsin to aid virus entry into the cell lines (WHO 2005d). Conventional cell culture takes up to two weeks but has a very high sensitivity. Cytopathic effects such as syncytia and intracytoplasmic basophilic inclusion bodies are observed. The presence of influenza virus can be ascertained using haemadsorption using guinea pig red blood cells (Weinberg 2005) or immunofluorescence on cultured cells. The latter can also be used to type the isolated virus. Immunofluorescence has a higher sensitivity in detection of positive cultures than haemadsorption.

Shell vial culture: Shell vial culture allows for diagnosis within 48 hours (Allwinn 2002). This is brought about by centrifugation of the inoculum onto the cell culture monolayer and the performance of immunofluorescence before a cytopathic effect can be observed. Shell vial culture can however be less sensitive than conventional culture (Weinberg 2005).

Laboratory animals

Ferrets are often used in research facilities as a model of human influenza infection but have no role in routine diagnosis.

Serology

Serology refers to the detection of influenza virus-specific antibodies in serum (or other body fluids).

Serology can either detect total antibodies or be class-specific (IgG, IgA, or IgM).

Different serological techniques are available for influenza diagnosis: haemagglutination inhibition (HI), compliment fixation (CF), enzyme immunoassays (EIA) and indirect immunofluorescence.

Serological diagnosis has little value in diagnosing acute influenza. In order to diagnose acute infection, an at least four-fold rise in titre needs to be demonstrate, which necessitates both an acute and a convalescent specimen. However it may have value in diagnosing recently infected patients.

Serology is also used to determine the response to influenza vaccination (Prince 2003).

Serology has greater clinical value in paediatric patients without previous exposure to influenza since previous exposure can lead to heterologous antibody responses (Steininger 2002).

Haemagglutination inhibition (HI)

HI assays are labour intensive and time consuming assays that require several controls for standardisation. However the assay reagents are cheap and widely available. Various red blood cells such as guinea pig, fowl and human blood group “O” erythrocytes are used. An 0.4–0.5% red blood cell dilution is generally used. Serum is pre-treated to remove non-specific haemagglutinins and inhibitors. A viral haemagglutinin preparation that produces visible haemagglutination (usually 4 haemagglutination units) is then pre-incubated with two-fold dilutions of the serum specimen. The lowest dilution of serum that inhibits haemagglutination is the HI titre. HI is more sensitive than complement fixation (Julkunen 1985, Prince 2003) and has the added advantage that it is more specific in differentiating between HA subtypes (Julkunen 1985).

Complement fixation (CF)

Complement fixation tests are based on the ability of antigen-antibody complexes to consume complement – which results in no compliment being available to lyse sensitised sheep red blood cells. These assays are labour intensive and necessitate controls for each procedure but reagents are cheap and widely available. CF assays are less sensitive than HI both in the diagnosis of acute infection and the determination of immunity after vaccination (Prince 2003).

Ezyme immuno assays (EIA)

EIAs are more sensitive than HI or CF assays (Bishai 1978, Julkunen 1985). Various commercial EIAs are available. Assays that detect IgG and IgA are more sensitive than IgM assays (Julkunen 1985) but are not indicative of acute infection.

Indirect immunofluorescence

Indirect immunofluorescence is not commonly used as a method to detect influenza virus antibodies.

Rapid tests

The clinical value of a diagnostic test for influenza is to a large extent dependent on the particular test’s turnaround time. The first diagnostic tests that were developed

for influenza diagnosis were virus isolation and serological assays. At that stage it took more than two weeks to exclude influenza infection. Although shell vial tests have reduced the turn-around time of isolation, they are not generally regarded as rapid tests.

The development of direct tests such as immunofluorescence enabled the diagnosis within a few hours (1 to 2 incubation and wash steps). Immunofluorescence tests however necessitate skilled laboratory workers and the availability of immunofluorescence microscopes.

The revolution in rapid diagnosis of influenza was brought about by the development of rapid antigen assays (most of which work on an EIA or immunochromatography principle). These assays enable the diagnosis of influenza within 10-30 minutes. Some of these tests are so easy to perform that even non-laboratory trained people can perform these tests in the clinic, which is referred to as bedside or point-of-care testing.

RT-PCR reactions that required a gel electrophoresis step were initially time consuming but the relatively recent development of real-time technology made RT-PCR diagnosis within about two hours possible. Although antigen assays are generally the most user-friendly, they are not as sensitive as direct immunofluorescence, isolation or RT-PCR.

Table 1 compares the characteristics of the different test methods available for influenza diagnosis.

Table 1: Comparison of test characteristics*

Test	Sensitivity	Turnaround time	Ease of performance	Affordability
<i>Direct detection</i>				
Rapid tests (EIA / chromatography)	-2	+2	+2	0
Immunofluorescence	0	+1	+1	+1
Gel electrophoresis RT-PCR	+2	0	-1	-2
Real-time RT-PCR	+2	+1	-1	-2
<i>Viral culture</i>				
Routine viral culture	+2	-2	-1	+2
Shell vial culture	+1	0	-1	+1
<i>Serology</i>				
EIA	+2	-2	+1	+1
Haemagglutination inhibition	+1	-2	-1	+2
Complement fixation	0	-2	-2	+2

*Relative criteria for favourability of tests (5 point ordinal scale)

-2: very unfavourable characteristic

-1: unfavourable characteristic

0: average characteristic

+1: favourable characteristic

+2: very favourable characteristic

Differential diagnosis of flu-like illness

Many different symptoms are described as influenza-like: fever, cough, nasal congestion, headache, malaise and myalgia. However no clear definition or uniformity in the use of the term “flu-like” exists.

During an epidemic the clinical symptoms of fever, cough, severe nasal symptoms and loss of appetite are highly predictive of influenza (Zambon 2001). However many other infections can present with influenza-like symptoms. These include viral, bacterial, mycoplasmal, chlamydial and fungal infections and also parasite infestations. Infections that could either be life-threatening also in the young and healthy, such as viral haemorrhagic fevers, or infections such as legionellosis that are life-threatening in at-risk groups such as the old-aged, can initially present with flu-like symptoms. Therefore it is important to consider a wide differential diagnosis which should be guided by the patient’s history, which includes travel, occupational exposure, contact with animals and sick individuals, history of symptoms as well as the local epidemiology of disease.

Diagnosis of suspected human infection with an avian influenza virus

Introduction

Accurate and rapid clarification of suspected cases of H5N1 infection by laboratory diagnosis is of paramount importance in the initiation and continuation of appropriate treatment and infection control measures. Isolation of virus from specimens of suspected cases of avian influenza should be conducted in specialised reference laboratories with at least Biosafety Level 3 facilities.

Specimen collection

Specimens for virus detection or isolation should be collected within 3 days after the onset of symptoms and rapidly transported to the laboratory. A nasopharyngeal aspirate, nasal swab, nasal wash, nasopharyngeal swab, or throat swab are all suitable for diagnosis. However a nasopharyngeal aspirate is the specimen of choice. In cases where patients are intubated, a transtracheal aspirates and a bronchoalveolar lavage can be collected.

At the same time, acute and convalescent serum samples should be collected for serological diagnosis (WHO 2005b).

Virological diagnostic modalities

Rapid identification of the infecting agent as an influenza A virus can be performed by ordinary influenza rapid tests that differentiate between types. However commercial rapid chromatographic methods have a sensitivity of only 70% for avian influenza compared to culture (Yuen 2005). Direct diagnosis of influenza H5N1 infection can be performed by indirect immunofluorescence on respiratory cells fixed to glass slides using a combination of influenza type A/H5-specific monoclonal antibody pool, influenza A type specific and influenza B type-specific monoclonal antibody pools as well as influenza A/H1 and an A/H3 specific monoclonal

antibody (available from WHO) and anti-mouse FITC for the detection step. This assay allows for the rapid differentiation of human H5 influenza infection from other influenza types and subtypes but cannot exclude H5N1 infection due to lack of sensitivity. Therefore culture and/ or RT-PCR that are more sensitive should also be performed.

Virus can be isolated in embryonated chicken eggs, Madin Darby canine kidney (MDCK) cells or Rhesus monkey kidney cells (LLC-MK2) (de Jong 2005, Yuen 2005). Other common cell lines such as Hep-2 or RD cells are also permissible to avian influenza A/H5 virus. Cytopathic effects are non-specific and influenza A virus infection of cells can be detected by immunofluorescence for nucleoprotein. HI of cell culture supernatant, H5-specific immunofluorescence (using monoclonal antibodies against H5) or RT-PCR can be used to subtype these viruses. Primers are available to detect both H5 and N1 genes of avian influenza by RT-PCR (WHO 2005c). H9-specific primers are also available (WHO 2005c).

Detection of Influenza A/H5 by real-time RT-PCR offers a rapid and highly sensitive method to diagnose H5N1 infection (Ng 2005).

Serology: A fourfold rise in titre from acute to convalescent specimens is also diagnostic of infection in patients that recovered (Yuen 2005).

Other laboratory findings

Leucopenia and especially lymphopenia (which has been shown to be a sign of poor prognosis in patients from Thailand), thrombocytopenia and moderately elevated transaminase levels are common findings (Beigel 2005).

New developments and the future of influenza diagnostics

A few trends in influenza diagnosis have been observed. The availability of anti-influenza drugs which must be given early in infection in order to be effective has emphasised the need for early diagnosis which stimulated the development of many EIA or immunochromatographic rapid tests with such low complexity that they enable bedside testing. Yet these tests' value is limited by their relatively low sensitivity especially for the diagnosis of avian influenza.

Real-time RT-PCR offers a highly sensitive and specific alternative. Technological developments are making real-time RT-PCR more widely available since instruments are becoming smaller, more efficient and user-friendly. Therefore real-time RT-PCR has already gained prominence in influenza pandemic preparedness since it will enable laboratories to make a rapid sensitive and specific diagnosis of human cases of avian influenza. The only remaining hurdle remains its relative high cost; but the highly competitive market has already made these tests more affordable.

Conclusion

Molecular diagnostic techniques play a more and more prominent role in laboratory diagnosis of influenza. Direct rapid tests have also become an important tool for investigating influenza-like illness.

Viral culture however remains important especially for reference laboratories since it is cheap, sensitive and enables characterisation of viruses. Furthermore unlike molecular testing it is “unbiased” and can detect the unexpected new strain.

Influenza serology’s main value lies in epidemiological investigations of yearly epidemics, avian to human transmissions and drug and vaccine trials. It has limited value for routine diagnosis.

We can thus conclude that virological diagnosis for influenza has value for the individual patient, epidemiological investigations and infection control. The appropriate selection of a particular test will be determined by the test characteristics and the specific diagnostic or public health needs.

A positive diagnostic test is the difference between someone with flu-like illness and a definite diagnosis of influenza or between a suspected human case of avian influenza and a confirmed case.

Useful Internet sources relating to Influenza Diagnosis

<http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5408a1.htm>

<http://www.fda.gov/cdrh/ovd/tips/rapidflu.html>

http://www.who.int/csr/disease/avian_influenza/guidelines/RapidTestInfluenza_web.pdf

http://www.who.int/csr/disease/avian_influenza/guidelines/humanspecimens/en/print.html

http://www.who.int/csr/disease/avian_influenza/guidelines/avian_labtests2.pdf

<http://www.who.int/csr/resources/publications/influenza/whocdscsrncs20025rev.pdf>

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Chapter 8: Clinical Presentation

Christian Hoffmann and Bernd Sebastian Kamps

Uncomplicated Human Influenza

After a short incubation period of 1–2(–4) days, onset of the disease is usually abrupt with typical systemic symptoms: high fever and chills, severe malaise, extreme fatigue and weakness, headache or myalgia, as well as respiratory tract signs such as non-productive cough, sore throat, and rhinitis (CDC 2005) (Tables 1 and 2). Among children, otitis media, nausea, and vomiting are also common (Peltola 2003). In rare cases, the initial presentation may be atypical (febrile seizures, Ryan-Poirier 1995; bacterial sepsis, Dagan 1984).

Table 1. Typical symptoms of uncomplicated influenza

Abrupt onset

Systemic: feverishness, headaches, myalgias (extremities, long muscles of the back; eye muscles; in children: calf muscles), malaise, prostration

Respiratory: dry cough, nasal discharge – may be absent in elderly people who may present with lassitude and confusion instead

Hoarseness, dry or sore throat often appear as systemic symptoms diminish

Croup (only in children)

Table 2: Frequency of baseline symptoms*

Symptom	(%)
Fever $\geq 37.8^{\circ}\text{C}$	68
Feverishness**	90
Cough	93
Nasal congestion	91
Weakness	94
Loss of appetite	92
Sore throat	84
Headache	91
Myalgia	94

*In 2,470 patients with laboratory-confirmed influenza (adapted from Monto 2000)

**Defined as the patient's subjective feeling that they had a fever or chill

The severity of clinical presentation varies from afebrile respiratory symptoms mimicking the common cold to severe prostration without major respiratory signs and symptoms, especially in the elderly. The severity of symptoms is related to the severity of the fever.

Fever and systemic symptoms typically last 3 days, occasionally up to 4–8 days, and gradually diminish; however, cough and malaise may persist for more than

2 weeks. Second fever spikes are rare. The physical findings are summarised in table 3. Full recovery may take 1–2 weeks, or longer, especially in the elderly.

Table 3. Physical findings of uncomplicated influenza

Fever: rapidly peaking at 38–40°C (up to 41°C, especially in children), typically lasting 3 days (up to 4–8 days), gradually diminishing; second fever spikes are rare.

Face: flushed

Skin: hot and moist

Eyes: watery, reddened

Nose: nasal discharge

Ear: otitis

Mucous membranes: hyperaemic

Cervical lymph nodes: present (especially in children)

Adults are infectious from as early as 24 hours before the onset of symptoms until about seven days thereafter. Children are even more contagious: young children can shed virus for several days before the onset of their illness (Frank 1981) and can be infectious for > 10 days (Frank 1981). Severely immunocompromised persons can shed influenza virus for weeks or months (Klimov 1995, Boivin 2002).

During non-epidemic periods, respiratory symptoms caused by influenza may be difficult to distinguish from symptoms caused by other respiratory pathogens (see Laboratory Findings). However, the sudden onset of the disease, fever, malaise, and fatigue are characteristically different from the common cold (Table 4).

Table 4. Influenza or common cold ?

Symptoms	Influenza	Cold
Fever	Usually high, lasts 3–4 days	Unusual
Headache	Yes	Unusual
Fatigue and/or weakness	Can last up to 2–3 weeks	Mild
Pains, aches	Usual and often severe	Slight
Exhaustion	Early and sometimes severe	Never
Stuffy nose	Sometimes	Common
Sore throat	Sometimes	Common
Cough	Yes	Unusual
Chest discomfort	Common and sometimes severe	Mild to moderate
Complications	Bronchitis, pneumonia; in severe cases life-threatening	Sinus congestion

Complications of Human Influenza

The most frequent complication of influenza is pneumonia, with secondary bacterial pneumonia being the most common form, and primary influenza pneumonia the most severe. In addition, mixed viral and bacterial pneumonia frequently occurs during outbreaks.

Influenza may exacerbate heart or lung diseases or other chronic conditions. Influenza infection has also been associated with encephalopathy (McCullers 1999, Morishima 2002), transverse myelitis, myositis, myocarditis, pericarditis, and Reye's syndrome.

Secondary Bacterial Pneumonia

Secondary bacterial pneumonia is most commonly caused by *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*. Typically, patients may initially recover from the acute influenza illness over 2 to 3 days before having rising temperatures again. Clinical signs and symptoms are consistent with classical bacterial pneumonia: cough, purulent sputum, and physical and x-ray signs of consolidation. Gram staining and culture of sputum specimens may determine the aetiology. Chronic cardiac and pulmonary disease predispose to secondary bacterial pneumonia, as does older age. Institution of an appropriate antibiotic regimen is usually sufficient for a prompt treatment response.

Primary Viral Pneumonia

Clinically, primary viral pneumonia presents as an acute influenza episode that does not resolve spontaneously. The clinical situation worsens with persistent fever, dyspnoea, and cyanosis. Initially, physical findings may be unimpressive. In more severe cases, diffuse rales may sometimes be present. At this stage, x-ray findings show diffuse interstitial infiltrates and acute respiratory distress syndrome (ARDS) with marked hypoxia. Viral titres are high in specimen cultures of respiratory secretions or lung tissue.

Primary influenza pneumonia with pulmonary haemorrhages was a prominent feature of the 1918 pandemic. In addition, pregnant women and individuals with cardiac disease (mitral stenosis) and chronic pulmonary disorders were found to be at increased risk during the 1957 pandemic.

Mixed Viral and Bacterial Pneumonia

Mixed influenza pneumonia has clinical features of both primary and secondary pneumonia. It most often occurs in patients with underlying chronic pulmonary or cardiovascular diseases. Some patients have a slowly progressive course, others may show a transient improvement in their condition, followed by clinical exacerbation. Treatment aims at eradicating the bacterial pathogens involved.

Exacerbation of Chronic Pulmonary Disease

Infectious pathogens have long been recognised as playing an important role in the pathogenesis of chronic respiratory disease (Monto 1978). In patients with chronic bronchitis, clinical influenza infection may lead to a permanent loss of pulmonary function. In children, influenza-induced asthma may continuously deteriorate during the first two days of illness and convalescence is typically longer (at least seven days) (Kondo 1991). Influenza virus is also implicated in the pathogenesis of asthma attacks in adults (Techtahl 1997).

Croup

Croup is a typical complication of influenza infection in children. The clinical picture of croup caused by influenza viruses may be more severe than that caused by parainfluenza viruses (Peltola 2002).

Failure of Recovery

In epidemic influenza outbreaks, severely compromised elderly people are at particular risk. Pneumonia and influenza death rates have ranged from fewer than ten to more than 600 per 100,000 among healthy versus chronically ill adults. In one study, the highest death rates (870 per 100,000) occurred in individuals with both cardiovascular and pulmonary disease (Barker 1982). More importantly, the risk of death may extend well beyond the first weeks after influenza complications. Some people may simply never recover from influenza complications – and eventually die from deterioration of underlying pulmonary, cardiovascular, or renal function (Saah 1986).

Myositis

Myositis is a rare complication of influenza B virus infection, and to a lesser extent influenza A. It has mainly been reported in children, with boys being more commonly affected than girls. The median interval between the onset of influenza and the onset of benign acute childhood myositis is 3 days (Agyeman 2004). The calf muscles are involved alone or together with other muscle groups in 69 % and 31 % of cases, respectively. Blood creatine phosphokinase concentration is generally elevated (Hu 2004). Symptoms usually resolve within 3 days and may rarely persist for a couple of weeks. When myositis occurs in elderly patients, it is important to distinguish influenzal myositis from other forms of myopathy (Oba 2000).

Cardiac Complications

Myocarditis is a rare event during influenza infection. In an unselected cohort of patients with serologically confirmed acute influenza infection (n=152), the prevalence of elevated creatine kinase levels was 12 %. Of note, cardiac troponin I and T levels were not raised in any of the patients. The authors concluded that the prevalence of myocarditis during acute influenza infection is substantially lower than previously thought, whereas skeletal muscle injury is relatively common (Greaves 2003).

In a study determining the frequency, magnitude, and duration of myocardial dysfunction in previously healthy young adult patients, abnormal electrocardiogram findings have been noted in 53 %, 33 %, 27 %, and 23 % of patients on days 1, 4, 11, and 28, respectively, but none of the findings were considered to be clinically significant. No patients had significant changes in the ejection fraction or abnormal wall motions. None of the patients had an elevated CK-MB index or troponin I level (Ison 2005).

Toxic Shock Syndrome

Toxic shock syndrome (TSS) can occur as a complication of influenza (CDC 1986, MacDonald 1987, Tolan 1993). One of the hallmarks of the disease is rapidly de-

veloping, severe and sometimes refractory hypotension (Chesney 1981). The TSS diagnosis is based on a clinical case definition (Reingold 1981), and toxin-producing *Staphylococcus aureus* can usually be demonstrated in sputum specimens.

The differential diagnosis of sudden shock in this clinical setting includes myocarditis and septic shock. The differentiation of these illnesses can be difficult, often requiring haemodynamic monitoring, serologic testing, and cultures from appropriate clinical specimens (CDC 1986).

Reye's Syndrome

Reye's syndrome is characterised by the combination of liver disease and non-inflammatory encephalopathy. It is a non-specific clinicopathological entity and a descriptive term which covers a group of heterogeneous disorders. It is almost always associated with previous viral infections, such as influenza, cold, or chickenpox. Differential diagnoses include encephalitis, meningitis, diabetes, drug overdose, poisoning, or psychiatric illness.

In influenza, Reye's syndrome is a serious complication that may occur in children, in particular with influenza B virus. There is a strong link between the administration of aspirin and Reye's syndrome (Starko 1980, Waldman 1982, Halpin 1983). When this association was recognised, the use of salicylates among children and teenagers with acute viral respiratory infections was discouraged. As a result, the incidence of Reye's syndrome markedly decreased (Barrett 1986).

In the first outbreak of avian influenza among humans in Hong Kong in 1997, one child died from influenza pneumonia, acute respiratory distress syndrome, Reye's syndrome, multiorgan failure, and disseminated intravascular coagulation (Claas 1998).

Complications in HIV-infected patients

The clinical presentation of influenza in patients infected with HIV is no different from other patient groups (Skiest 2001). Unusual clinical manifestations are rare and the rate of pulmonary complications is similar to that of HIV-negative patients. However, in small series, the hospitalisation rate has been reported to be higher than that commonly seen in HIV-negative individuals (Skiest 2001, Fine 2001). Only HAART seems to be able to reduce the number of influenza-associated hospitalisations (Neuzil 2003).

Influenza may be less benign in patients with AIDS, i.e., in more advanced stages of immunosuppression. In these patients, influenza has been associated in the US with excess death rates substantially higher than that of the general population and comparable to the general population aged 65 years and older (Lin 2001).

Avian Influenza Virus Infections in Humans

Avian influenza virus strains have only recently been identified as the cause of human disease. For most of these, the clinical manifestations in humans are mild. In 1996, an avian H7 virus was isolated from a woman with conjunctivitis (Kurtz

1996). In 1999, a **H9N2** strain was isolated in Hong Kong from two children with mild influenza symptoms (Peiris 1999, Horimoto 2001). 4 years later, in an outbreak of a highly pathogenic subtype **H7N7** strain in the Netherlands, conjunctivitis was the prominent feature among 89 persons infected; only 7 individuals had an influenza-like illness that was generally mild. However, one fatal case of pneumonia occurred in a man (Fouchier 2004): two days after visiting a poultry farm affected by avian influenza, the 57-year-old veterinarian developed malaise, headache and fever. Eight days later he developed pneumonia, and his condition then deteriorated. He died four days later of acute pneumonia.

The only avian influenza strain to cause repeatedly severe disease in humans is the **H5N1** serotype, first diagnosed in humans in Hong Kong in 1997 (CDC 1997, Yuen 1998). So far, the number of human cases has fortunately been relatively low (152 as of 23 January 2006), but the case-fatality rate is high (83/152) (WHO 20051223). The clinical manifestations of influenza H5N1 infection in humans is not well-defined as current knowledge is based on the description of a few hospitalised patients. The spectrum ranges from asymptomatic infection (Katz 1999, Buxton Bridges 2000, Thorson 2006) to fatal pneumonitis and multiple organ failure.

Presentation

Initial symptoms of H5N1 influenza may include fever (typically $> 38^{\circ}\text{C}$), headache, malaise, myalgia, sore throat, cough, and rhinitis (although upper respiratory symptoms may be absent), gastrointestinal manifestations and conjunctivitis (Yuen 1998, Chan 2002). All these symptoms are non-specific and may also be associated with the currently circulating human influenza virus subtypes, H1N1 and H3N2. In two reports, diarrhoea (Hien 2004) was a prominent feature along with shortness of breath (Hien 2004, Chotpitayasunondh 2005). Watery diarrhoea may be present well before pulmonary symptoms develop (Apisarnthanarak 2004). Another report describes a four-year-old boy with severe diarrhoea, followed by seizures, coma, and death, suggesting the clinical diagnosis of encephalitis – avian influenza H5N1 was later detected in cerebrospinal fluid, faecal, throat, and serum specimens (de Jong 2005).

Laboratory findings of patients with severe avian influenza H5N1 include leucopenia, lymphopenia, impaired liver function with elevated liver enzymes, prolonged clotting times, and renal impairment. The lymphocyte count appears to be the most valuable parameter for identification of patients who are at risk of progression to severe illness (Chan 2002).

Clinical Course

As of December 2005, about half of the patients diagnosed with clinical avian H5N1 influenza infection have died. Most of these patients had severe disease on admission to hospital. In patients with respiratory failure and fatal outcome, dyspnoea developed after a median of 5 days (range 1–16) in one series (Chotpitayasunondh 2005). Abnormal chest radiographs include interstitial infiltration, patchy lobar infiltrates in a variety of patterns (single lobe, multiple lobes, unilateral or bilateral distributions). Finally, the radiographic pattern progresses to a diffuse bilateral ground-glass appearance, with clinical features compatible with ARDS (Chotpitayasunondh 2005). In the report from Vietnam, major x-ray abnor-

malities include extensive bilateral infiltration, lobar collapse, focal consolidation, and air bronchograms. All patients had dramatic worsening of findings on chest radiography during hospitalisation. The median time from onset of fever to ARDS was 6 days (range 4–13) in one series (Chotpitayasunondh 2005). Pneumothorax may develop in patients during mechanical ventilation (Hien 2004). Pleural effusions are uncommon.

There is conflicting information as to the risk factors associated with severe disease and fatal outcome. In the 1997 outbreak in Hong Kong, the factors associated with severe disease included older age, delay in hospitalisation, lower respiratory tract involvement, and a low total peripheral white blood cell count or lymphopenia on admission (Yuen 1998). In this report, patients aged below 6 years usually had a self-limiting acute respiratory disease with fever, rhinorrhoea, and sore throat. In contrast, recent avian H5N1 infections have caused high rates of death among infants and young children (Chotpitayasunondh 2005). The numbers reported are too small to understand whether local factors – i.e., time between onset of symptoms and admission to hospital – or viral virulence factors are responsible for these differences. As H5N1 strains have evolved over the past 10 years (Webster 2006), clinical features of avian influenza infection in humans may well have different characteristics over time.

The progression of severe H5N1 infection seems to be different from that of severe diseases observed during earlier influenza pandemics. None of the patients with severe disease reported from Hong Kong (Yuen 1998) and Vietnam (Hien 2004) had evidence of secondary bacterial pneumonia, suggesting that the fatal outcome was due to an overwhelming primary viral pneumonia. This feature is reminiscent of the 1918 pandemic and may pathogenetically be due to a "cytokine storm" (Barry 2004).

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Chapter 9: Treatment and Prophylaxis

C. Hoffmann, S. Korsman and B.S. Kamps

Introduction

Most patients with uncomplicated human influenza, especially adolescents and young adults, can be treated symptomatically and need no specific intervention. In the elderly, however, treatment with antiviral drugs is a good option. These drugs should further be considered for high-risk individuals, especially patients with underlying medical conditions, as well as in a number of special situations.

Neuraminidase inhibitors are effective against all variants that have caused disease in humans, including the virus of the 1918 pandemic (Tumpey 2005). In human H5N1 influenza, treatment with an oral neuraminidase inhibitor, oseltamivir, seems to be effective in some cases, but may fail in others. Recently, resistant strains have been reported (de Jong 2005). In addition, the dosage and duration of treatment appear to be different in severe H5N1 cases.

In the case of a future pandemic, antiviral drugs may play an important role in the early phase, when vaccines against the new strain are not yet available or as long as the available vaccine is in short supply.

Antiviral Drugs

Of the four antiviral drugs currently available for the treatment of influenza A infection (two neuraminidase inhibitors and two M2 ion channel inhibitors), only the neuraminidase inhibitors oseltamivir and zanamivir are also active against influenza B. All drugs are most effective if started within a few hours of the onset of symptoms and are generally licensed for use within 48 hours of the first symptoms. They can modify the severity of illness, as well as reducing the intensity of influenza symptoms and decreasing the duration of illness by about 1 to 3 days. However, the extent to which antiviral treatment leads to a reduction of serious complications and hospitalisation is still subject to debate. Treatment success is, in part, a variable of the time between the onset of symptoms and the beginning of antiviral treatment: the sooner after onset treatment begins, the better.

The neuraminidase inhibitors, oseltamivir and zanamivir, have fewer side effects than the M2 ion channel inhibitors rimantadine and amantadine, and drug resistance seems to develop less frequently. The clinical pharmacology, adverse effects and resistance profiles of these drugs are discussed in detail in the [Drugs](#) chapter.

The neuraminidase inhibitor, oseltamivir (Tamiflu[®]), is currently the drug of choice for the treatment of human H5N1 influenza.

Neuraminidase Inhibitors

These drugs – introduced in 1999 and 2000 – interfere with the normal function of the influenza neuraminidase by mimicking sialic acid, the natural substrate of the

neuraminidase (Varghese 1992, Varghese 1995). The viral neuraminidase is responsible for cleaving sialic acid residues on newly formed virions, playing an essential role in their release and facilitating virus spread within the respiratory tract. When exposed to neuraminidase inhibitors, the influenza virions aggregate on the surface of the host cell, limiting the extent of infection within the mucosal secretions (McNicholl 2001) and reducing viral infectivity (see Figure at <http://content.nejm.org/cgi/content/full/353/13/1363/F1>). Experimental evidence further suggests that influenza neuraminidase may be essential at the early stage of virus invasion of the ciliated epithelium of human airways (Matrosovich 2004). The design of the neuraminidase inhibitors was a result of the analysis of the three-dimensional structure of influenza neuraminidase which disclosed the location and structure of the catalytic site (Colman 1983).

Numerous **treatment** studies in healthy adults have shown that neuraminidase inhibitors, when taken within 36 to 48 hours after the onset of symptoms, decrease the symptomatic illness by one or two days (Hayden 1997, Monto 1999, Treanor 2000, Nicholson 2000, Hedrick 2000, Cooper 2003, Whitley 2001, Aoki 2003). Early initiation of treatment is decisive for treatment efficacy (Aoki 2003, Kawai 2005). When started within the first 12 hours following the onset of fever, neuraminidase inhibitors shortened the illness by more than three days, in comparison to treatment that was started at 48 hours. The duration of fever, severity of symptoms, and time to return to normal activity also correlated with the time of initiation of antiviral intervention.

A study in Canadian long-term care facilities showed that older nursing home residents who were treated with oseltamivir within 48 hours after the onset of symptoms were less likely to be prescribed antibiotics, to be hospitalised, or to die (Bowles 2002). Side effects were rare (4.1 %), the most common being diarrhoea (1.6 %), cough (0.7 %), confusion (0.5 %) and nausea (0.5 %). Another study suggested that oseltamivir treatment of influenza illness reduces lower respiratory tract complications, antibiotic use, and hospitalisation in both healthy and “at-risk” adults (Kaiser 2003).

Prevention trials have shown that neuraminidase inhibitors administered prophylactically reduce the risk of developing influenza by 60-90 % when given at the start of the influenza outbreak (Monto 1999b, Cooper 2003). When administered prophylactically to household contacts of an influenza index case, protective efficacy against clinical influenza was generally > 80 % (Hayden 2000, Kaiser 2000, Welliver 2001, Monto 2002).

Neuraminidase inhibitors are generally well-tolerated. Transient gastrointestinal disturbance (nausea, vomiting) is the major **adverse effect** of oseltamivir. In particular, the observed safety profile of oseltamivir and zanamivir compares favourably with the M2 inhibitors rimantadine and amantadine (Freund 1999, Doucette 2001).

Rarely, with oseltamivir, serious skin/hypersensitivity reactions may occur, and patients should, therefore, be cautioned to stop taking oseltamivir and contact their healthcare providers if they develop a severe rash or allergic symptoms (FDA 2005). Bronchospasm and a decline in lung function (FEV₁ or peak expiratory flow) have been reported in some patients with underlying pulmonary conditions, such as asthma or chronic obstructive pulmonary disease, on zanamivir. Zanamivir is therefore not generally recommended for the treatment of patients with underly-

ing airways disease, and should also be discontinued in patients who develop bronchospasm or who have a decline in respiratory function (Relenza 2003).

The potential for drug-drug **interactions** is low, both for oseltamivir and zanamivir. In oseltamivir, competitive inhibition of excretion by the renal tubular epithelial cell anionic transporter may occur. Probenecid may more than double systemic exposure to oseltamivir carboxylate (Hill 2002).

Naturally occurring virus strains **resistant** to neuraminidase inhibitors are believed not to exist in human influenza A (McKimm-Breschkin 2003). In vitro, the NA mutations E119V, R292K, H274Y, and R152K were associated with resistance to oseltamivir (McKimm-Breschkin 2003). Some mutations, i.e., the R292K and H274Y mutation, lead to a functionally defective enzyme with compromised viral fitness, and it has been suggested that viruses carrying these mutations are unlikely to be of significant clinical consequence in man (Tai 1998, Carr 2002, Ives 2002, Herlocher 2004). However, a recent report describes a resistant H5N1 strain carrying the H274Y mutation causing viremia in two patients who subsequently died from avian influenza (de Jong 2005). Zanamivir seems to retain *in vitro* activity against some oseltamivir-resistant strains (McKimm-Breschkin 2003, Mishin 2005).

Following clinical use, the incidence of development of resistant strains is lower among adults and adolescents older than 13 years, than among children. One study found neuraminidase mutations in strains from 9/50 children (18 %) taking oseltamivir. (Kiso 2004). These findings are reason for concern, since children are an important transmission vector for the spread of influenza virus in the community. In the case of an H5N1 pandemic, the frequency of resistance emergence during oseltamivir treatment of H5N1 paediatric patients is uncertain, but it is likely to be no less than that observed in children infected with currently circulating human influenza viruses (Hayden 2005).

Neuraminidase inhibitors are effective against the virus that caused the 1918 pandemic (Tumpey 2002).

Indications for the Use of Neuraminidase Inhibitors

Oseltamivir (Tamiflu®) and zanamivir (Relenza®) are currently licensed for the **treatment** of influenza A and B. They should be used only when symptoms have occurred within the previous 48 hours and should ideally be initiated within 12 hours of the start of illness.

In addition, oseltamivir – but not zanamivir (with the exception of two countries) – is also licensed for **prophylaxis** when used within 48 hours of exposure to influenza and when influenza is circulating in the community; it is also licensed for use in exceptional circumstances (e.g. when vaccination does not cover the infecting strain) to prevent an influenza epidemic.

Oseltamivir and zanamivir seem to have similar efficacy, but they differ in their modes of delivery and tolerability. Zanamivir is delivered by inhalation and is well tolerated; however, children, especially those under 8 years old, are usually unable to use the delivery system appropriately and elderly people may have difficulties, too (Digory 2001). Oseltamivir is taken in the form of a pill but may produce nausea and vomiting in some patients.

M2 Ion Channel Inhibitors

Amantadine and rimantadine are tricyclic symmetric adamantanamines. In the 1960s it was discovered that they inhibited strains of influenza (Stephenson 2001). They are active only against influenza A virus (influenza B does not possess an M2 protein), have more side effects than neuraminidase inhibitors, and may select for readily transmissible drug-resistant viruses.

M2 inhibitors block an ion channel formed by the M2 protein that spans the viral membrane (Hay 1985, Sugrue 1991) and is required for viral uncoating (for more details see the **Drugs** chapter). Both drugs are effective as **treatment** if started within 24 hours of illness onset, reducing fever and symptoms by 1–2 days (Wingfield 1969, Smorodintsev 1970, van Voris 1981).

Daily **prophylaxis** during an influenza season reduces infection rates by 50–90 % (Dawkins 1968, Dolin 1982, Clover 1986). Post-exposure prophylaxis of households seems problematic, though. In one study, rimantadine was ineffective in protecting household members from influenza A infection (Hayden 1989).

Gastrointestinal symptoms are the major **side effects** associated with amantadine and rimantadine. In addition, amantadine has a wide range of toxicity which may be in part attributable to the anticholinergic effects of the drug. In addition, minor reversible CNS side effects may occur during a 5-day treatment in up to one third of patients (van Voris 1981). The same frequency of side effects was found when the drug was tested in young healthy volunteers over a four-week period. Among 44 individuals, side effects (dizziness, nervousness, and insomnia) were well tolerated by most subjects, but 6 volunteers discontinued amantadine because of marked complaints. Cessation of side effects occurred in more than half of those continuing amantadine. 16 volunteers had decreased performance in sustained attention tasks (Bryson 1980). When studied in 450 volunteers during an outbreak of influenza A, the prophylactic effects of rimantadine and amantadine were comparable. Influenza-like illness occurred in 14 % of the rimantadine group and in 9 % of the amantadine group (Dolin 1982). Withdrawal from the study because of central nervous system side effects was more frequent in the amantadine (13 %) than in the rimantadine group (6 %).

The potential for drug **interactions** is greater for amantadine, especially when co-administered with central nervous system stimulants. Agents with anticholinergic properties may potentiate the anticholinergic-like side effects of amantadine. For more details see the chapter, “**Drugs**”.

Point mutations in the M gene lead to amino acid changes in the transmembrane region of the M2 protein and may confer high-level **resistance** to amantadine. The genetic basis for resistance appears to be single amino acid substitutions at positions 26, 27, 30, 31 or 34 in the transmembrane portion of the M2 ion channel (Hay 1985). The mutants are as virulent and transmissible as the wild-type virus. In an avian model, they were also genetically stable, showing no reversion to the wild-type after six passages in birds over a period of greater than 20 days (Bean 1989). Such strains may develop in up to one third of patients treated with amantadine or rimantadine; in immunocompromised individuals the percentage may even be higher (Englund 1998). Drug-resistant influenza A virus (H3N2) can be obtained from rimantadine-treated children and adults as early as 2 days after starting treatment (Hayden 1991). Some H5N1 strains which have been associated with human

disease in Southeast Asia are resistant against amantadine and rimantadine (Peiris 2004, Le 2005), while isolates from strains circulating in Indonesia and, more recently, in China, Mongolia, Russia, Turkey and Romania are amantadine sensitive (Hayden 2005).

Recently, adamantanes have come under pressure, since it was discovered that 91 % of influenza A H3N2 viruses, isolated from patients in the US during the current influenza season, contained an amino acid change at position 31 of the M2 protein, which confers resistance to amantadine and rimantadine. On the basis of these results, the Centre for Disease Control recommended that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza A in the United States for the remainder of the 2005–06 influenza season (CDC 2006). Some authors have suggested that the use of amantadine and rimantadine should be generally discouraged (Jefferson 2006).

Indications for the Use of M2 Inhibitors

Comparative studies indicate that rimantadine is tolerated better than amantadine at equivalent doses (Stephenson 2001). The advantage of amantadine is that it is cheap, 0.50 €/day in some European countries, compared to 5 €/day for rimantadine and 7 €/day for oseltamivir.

Treatment of “Classic” Human Influenza

In uncomplicated cases, bed rest with adequate hydration is the treatment of choice for most adolescents and young adult patients. If needed, treatment with acetylsalicylic acid (0.6–0.9 g every 3–4 hours) may be considered – headache, fever, and myalgia usually improve within hours. However, salicylates must be avoided in children of 18 years or younger because of the association of salicylate use and Reye’s syndrome. In these cases, acetaminophen or ibuprofen are common alternatives.

Nasal obstruction can be treated with sprays or drops, and cough with water vaporisation. Cough suppressants are needed only in a minority of patients. After the fever subsides, it is important to return to normal activity gradually. This is particularly true for patients who have had a severe form of the disease.

Antibiotic treatment should be reserved for the treatment of secondary bacterial pneumonia. Ideally, the choice of the drug should be guided by Gram staining and culture of respiratory specimens. In daily practice, however, the aetiology cannot always be determined, and so treatment is empirical, using antibacterial drugs effective against the most common pathogens in these circumstances (most importantly *S. pneumoniae*, *S. aureus*, and *H. influenzae*).

In more severe cases, supportive treatment includes fluid and electrolyte control, and finally supplemental oxygen, intubation, and assisted ventilation.

For more detailed information about the management of human H5N1 influenza, please see below.

Antiviral Treatment

Oseltamivir is indicated for the treatment of uncomplicated acute illness due to influenza infection in patients aged 1 year and older, who have been symptomatic for no more than 2 days. The recommended duration of treatment with oseltamivir is 5 days (but may be longer in severe H5N1 infection). A 7-day course of oseltamivir is also indicated for the prophylaxis of influenza in the same age group (EU: \geq 13 years).

Zanamivir is indicated for the treatment of uncomplicated acute illness due to influenza infection in patients aged 7 years and older and who have been symptomatic for no more than 2 days. With the exception of two countries, zanamivir has not been licensed for prophylactic use. The treatment duration is usually 5 days.

Rimantadine and amantadine are ineffective against the influenza B virus and are, therefore, indicated for prophylaxis and treatment of illness caused by influenza A virus only. To reduce the emergence of antiviral drug-resistant viruses, amantadine or rimantadine treatment should be discontinued as soon as clinically warranted, typically after 3–5 days of treatment or within 24–48 hours after the disappearance of signs and symptoms (CDC 2005).

Please note, that in the US, the CDC has recommended that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza A in the United States for the remainder of the 2005–06 influenza season (CDC 2006).

Antiviral Prophylaxis

Several studies have shown neuraminidase inhibitors to be effective in preventing clinical influenza in healthy adults following exposure to close contacts (Hayden 2000, Welliver 2001, Hayden 2004). They have also been used in seasonal prophylaxis (Monto 1999, Hayden 1999). In all these studies, neuraminidase inhibitors are 70 to 90 percent effective in preventing clinical disease caused by influenza A and B infection. With the exception of two countries, oseltamivir is the only neuraminidase inhibitor currently approved for prophylactic use. The adamantanes may be considered for prophylaxis if the circulating strain is influenza A.

Cost, compliance, and potential side effects must all be considered when deciding on the timing and duration of antiviral prophylaxis against influenza infection. To be effective as seasonal prophylaxis, the drugs should be taken throughout the entire period of a community outbreak, generally over 6 weeks. This approach might not be cost-effective, especially when compared to annual vaccinations (Patriarca 1989).

In a pandemic situation, there may even be fewer opportunities for prophylaxis if the next pandemic strain is resistant to M2 inhibitors (as was the case with certain serotypes of the H5N1 strain circulating in Southeast Asia in 2004 and 2005), and if neuraminidase inhibitors continue to be in short supply. If this happens, most of the available drug will probably be reserved for treatment, and prophylaxis might be limited to target groups with enhanced risk of exposure (health personnel, etc.).

In seasonal influenza, prophylaxis should be considered in the following situations (adapted from [CDC 2005](#)):

- Persons at high-risk who are vaccinated after influenza activity has begun

When influenza vaccine is administered while influenza viruses are circulating, chemoprophylaxis for 2 weeks should be considered for persons at high risk. Children aged < 9 years, receiving influenza vaccine for the first time, may require 6 weeks of prophylaxis (i.e., prophylaxis for 4 weeks after the first dose of vaccine and an additional 2 weeks of prophylaxis after the second dose).
- Persons who provide care to those at high risk

Healthcare personnel, if infected with influenza virus, can spread the disease. During the peak of influenza activity, prophylaxis with antiviral drugs can be considered for unvaccinated persons who have frequent contact with persons at high risk. Persons with frequent contact include employees of hospitals, clinics, and chronic-care facilities, household members, visiting nurses, and volunteer workers. If an outbreak is caused by a variant strain of influenza that might not be controlled by the vaccine, chemoprophylaxis should be considered for all such persons, regardless of their vaccination status.
- Persons who have immune deficiencies

Chemoprophylaxis can be considered for persons at high risk who are expected to have an inadequate antibody response to the influenza vaccine. This category includes persons infected with HIV, chiefly those with advanced HIV disease.
- Other persons

Chemoprophylaxis throughout the influenza season or during peak influenza activity might be appropriate for persons at high risk who should not be vaccinated.
- Institutions that house persons at high risk

There are several lines of evidence that institution-wide prophylaxis in nursing homes, given as soon as possible after influenza activity is detected, might be a valuable addition to institutional outbreak-control strategies (Peters 2001, Bowles 2002, [Monto 2004](#)). When confirmed or suspected outbreaks of influenza occur, chemoprophylaxis should, therefore, be started as early as possible, administered to all residents, regardless of whether they received influenza vaccinations during the previous fall, continued for a minimum of 2 weeks. If surveillance indicates that new cases continue to occur, chemoprophylaxis should be continued until approximately 1 week after the end of the outbreak. The dosage for each resident should be determined individually. Chemoprophylaxis also can be offered to unvaccinated staff who provide care to persons at high risk. Prophylaxis should be considered for all employees, regardless of their vaccination status, if the outbreak is caused by a variant strain of influenza that is not well-matched by the vaccine.

Special Situations

Children

Oseltamivir: children 1 to 12 years of age clear the active metabolite oseltamivir carboxylate at a faster rate than older children and adults, resulting in lower exposure. Increasing the dose to 2 mg/kg twice daily results in drug exposures comparable to the standard 1 mg/kg twice daily dose used in adults (Oo 2001). Infants as young as 1 year old can metabolise and excrete oseltamivir efficiently (Oo 2003), but in younger children, use of oseltamivir is contraindicated (FDA 2005).

Zanamivir: In the EU, zanamivir is approved for use in children aged 12 years or older (US: 7 years).

Amantadine, rimantadine: Given the relatively low efficacy and the high risk of developing gastrointestinal and CNS adverse effects, the authors do not recommend administration of amantadine or rimantadine to children.

Impaired Renal Function

Oseltamivir: the terminal plasma elimination half-life is 1.8 h in healthy adults. In patients with renal impairment, metabolite clearance decreases linearly with creatinine clearance, and averages 23 h after oral administration in individuals with a creatinine clearance < 30 ml/min (Doucette 2001). A dosage reduction to 75 mg once daily is recommended for patients with a creatinine clearance < 30 ml/min (1.8 l/h) (He 1999); in prophylaxis, a dosage of 75 mg every other day is recommended. No treatment or prophylaxis dosing recommendations are available for patients on renal dialysis treatment.

Zanamivir: the manufacturer declares that there is no need for dose adjustment during a 5-day course of treatment for patients with either mild-to-moderate or severe impairment in renal function (Relenza).

Rimantadine: renal insufficiency results in increased plasma concentrations of rimantadine metabolites. Haemodialysis does not remove rimantadine. A reduction to 100 mg/day is recommended in patients with a creatinine clearance < 10 ml/min. Supplemental doses on dialysis days are not required (Capparelli 1988). In patients with less severe renal insufficiency, and in older persons, rimantadine should be monitored for adverse effects.

Amantadine: a dose reduction is recommended for individuals > 60 years and with a creatinine clearance < 40 ml/min. Guidelines for amantadine dosage on the basis of creatinine clearance are located in the [package insert](#). Patients should be observed carefully for adverse reactions. In these cases, consider further dose reduction or discontinuation of the drug. Amantadine is not removed by haemodialysis.

Impaired Liver Function

Oseltamivir: the metabolism of oseltamivir is not compromised in patients with moderate hepatic impairment, and dose adjustment is not required in these patients (Snell 2005).

Zanamivir: has not been studied in persons with hepatic dysfunction.

Rimantadine: for persons with severe hepatic dysfunction, a dose reduction of rimantadine is recommended.

Amantadine: adverse reactions to amantadine have only rarely been observed among patients with liver disease.

Seizure Disorders

Seizures (or seizure-like activity) have rarely been reported among patients with a history of seizures who were not receiving anticonvulsant medication while taking amantadine or rimantadine.

Pregnancy

All drugs mentioned above should only be used during pregnancy if the potential benefit justifies the potential risk to the foetus (Pregnancy Category C).

Treatment of Human H5N1 Influenza

Experience with the treatment of H5N1 disease in humans is limited – until 8 March 2006, 175 confirmed cases had been reported to the WHO (WHO 2006), and clinical reports published to date include only a few patients (Yuen 1998, Chan 2002, Hien 2004, Chotpitayasunondh 2005, WHO 2005, de Jong 2005).

Based on current data, the treatment of influenza disease caused by the currently circulating H5N1 strains might be somewhat different from the treatment of “classical” influenza (WHO 2006b). However, it should be noted that current recommendations are preliminary and modifications are likely as new data come in:

- Patients with suspected H5N1 influenza should promptly receive a neuraminidase inhibitor pending the results of laboratory testing (WHO 2005).
- Oseltamivir (Tamiflu[®]) is currently regarded as the drug of choice.
- Consider increasing the dose of oseltamivir in severe disease (150 mg twice daily in adults) and continue treatment for longer periods (7–10 days or longer) (WHO 2005, WHO 2006b).
- Resistance may occur and precede clinical deterioration (de Jong 2005).
- Treatment with oseltamivir may be beneficial even when initiated as late as 8 days after the onset of symptoms, if there is evidence of ongoing viral replication (WHO 2005, de Jong 2005).

Corticosteroids have frequently been used, with conflicting results. In one series, six of the seven patients who were treated with corticosteroids died (Hien 2004). Ribavirin, interferon alpha and other immunomodulatory drugs have all been used, but without convincing results.

In severe cases, ventilatory support and intensive care may be needed within days of admission (Hien 2004, Chotpitayasunondh 2005).

Transmission Prophylaxis

As soon as a case of human H5N1 infection is suspected, precautions need to be taken to minimise nosocomial spread. If the diagnosis is confirmed, possible contacts of the index case must be identified to facilitate early intervention with antiviral therapy, in order to reduce morbidity and mortality and limit further spread of the disease (WHO 2004).

General Infection Control Measures

Infection control measures include the application of standard precautions (Garner 1996) to all patients receiving care in hospitals. If the diagnosis of H5N1 influenza infection is being considered on the basis of clinical features, additional precautions should be implemented until the diagnosis can be ruled out.

Special Infection Control Measures

Influenza virus is transmitted by droplets and fine droplet nuclei (airborne). In addition, transmission by direct and indirect contact is also possible. Although there is currently no evidence that the H5N1 virus is transmitted among humans, the WHO recommends the following precautions (WHO 2004):

- Use of high-efficiency masks in addition to droplet and contact precautions.
- Patients should be housed in a negative pressure room.
- Patients should be isolated to a single room. If a single room is not available, cohort patients separately in designated multi-bed rooms or wards.
- Patient beds should be placed more than 1 metre apart and preferably be separated by a physical barrier (e.g. curtain, partition).

To protect healthcare workers (HCWs) and other hospital personnel, the following recommendations have to be followed (WHO 2004):

- HCWs should protect themselves with a high efficiency mask (European CE approved respirators or US NIOSH certified N-95), gown, face shield or goggles, and gloves. The use of masks by healthcare workers in pandemic settings has recently been clarified (WHO 2005b). A surgical mask, when consistently used, may also reduce the risk of infection, but not significantly (Loeb 2004).
- Limit the number of HCWs who have direct contact with the patient(s); these HCWs should not look after other patients.
- The number of other hospital employees (e.g. cleaners, laboratory personnel) with access to the environment of these patients should also be limited.
- Designated HCWs should all be properly trained in infection control precautions. Restrict the number of visitors and provide them with appropriate personal protective equipment and instruct them in its use.
- Ask HCWs with direct patient contact to monitor their own temperature twice daily and to report any febrile event to hospital authorities. HCWs who have a fever > 38°C, and who have had direct patient contact, should be treated immediately.

- Offer post-exposure prophylaxis (for example, oseltamivir 75 mg daily orally for 7 days) to any HCW who has had potential contact with droplets from a patient without having had adequate personal protective equipment.
- HCWs who are unwell should not be involved in direct patient care since they are more vulnerable and may be more likely to develop severe illness when exposed to influenza A (H5N1) viruses.
- Dispose of waste properly by placing it in sealed, impermeable bags which should be clearly labelled “Biohazard” and incinerated. Linen and reusable materials that have been in contact with patients should be handled separately and disinfected.

Contact Tracing

Identify contacts as well as those persons who may have been exposed to the common source of infection. Contacts are persons who have shared a defined setting (household, extended family, hospital or other residential institution, military barracks or recreational camps) with a person in whom the diagnosis of influenza A(H5N1) is being considered while this person was in his or her infectious period (i.e. from 1 day prior to the onset of symptoms to 7 days after the onset of symptoms, or to the date prescribed by national public health authorities, or to the date indicated in the section “Discharge policy”) (WHO 2004).

These persons should be monitored for 7 days following the last exposure to the implicated patient, or to the common source, and asked to check their temperature twice daily. If a person who is being monitored develops fever ($> 38^{\circ}\text{C}$) and a cough or shortness of breath, he or she should be treated immediately (WHO 2004).

Discharge policy

The WHO recommends that infection control precautions for adult patients remain in place for 7 days after resolution of the fever. Previous human influenza studies have indicated that children younger than 12 years can shed virus for 21 days after the onset of illness. Therefore, infection control measures for children should ideally remain in place for this period (WHO 2004).

Where this is not feasible (because of a lack of local resources), the family should be educated on personal hygiene and infection control measures (e.g. hand-washing and use of a paper or surgical mask by a child who is still coughing). Children should not attend school during this period (WHO 2004).

Global Pandemic Prophylaxis

There is some evidence that containment and elimination of an emergent pandemic influenza strain at the point of origin is possible using a combination of antiviral prophylaxis and social distance measures (Ferguson 2005). The authors used a simulation model of influenza transmission in Southeast Asia to evaluate the potential effectiveness of targeted mass prophylactic use of antiviral drugs, and predicted that a stockpile of 3 million courses of antiviral drugs should be sufficient for elimination.

The WHO has recently started creating an international stockpile of antiviral drugs to be dispatched to the region of an emerging influenza pandemic (WHO 20000824). If the pandemic cannot be contained at its source, rapid intervention might at least delay international spread and gain precious time. For this strategy to work, a number of key criteria must be met to reach a high probability of success (Ferguson 2005):

1. rapid identification of the original case cluster,
2. rapid, sensitive case detection and delivery of treatment to targeted groups, preferably within 48 h of a case arising,
3. effective delivery of treatment to a high proportion of the targeted population, preferably > 90 %,
4. sufficient stockpiles of drugs, preferably 3 million or more courses of oseltamivir (the WHO disposes currently of this stockpile),
5. population co-operation with the containment strategy and, in particular, any social distance measures introduced,
6. international co-operation in policy development, epidemic surveillance and control strategy implementation.

It should be noted that the idea of stopping a pandemic at its source or delaying its international spread, is an attractive, but as yet untested hypothesis. So far, no attempt has ever been made to alter the natural course of a pandemic once it has emerged in the human population. The logistic issues involved in delivering the drug to large populations are considerable. In addition, the first pandemic viral strains should not be highly contagious, and the virus should be limited to a small geographical area. There are many “ifs”, and the outcome is all but certain. Nevertheless, given the potentially catastrophic consequences of an influenza pandemic, the WHO’s strategy of stockpiling antiviral drugs for rapid and early intervention is one of the numerous precious pieces of global pandemic preparedness planning.

Conclusion

The introduction of neuraminidase inhibitors was an important step for the more efficient control of human influenza infection. Today, neuraminidase inhibitors are the only drugs effective against recently isolated highly pathogenic avian influenza viruses in humans. However, reports on highly drug-resistant H5N1 strains underline the experience we have had with other viral infections such as HIV: we never have enough drugs to treat our patients and we will always need new and better ones. Great efforts lie ahead of us to develop more drugs and maybe even supervaccines that include antigens present in all subtypes of influenza virus, that do not change from year to year, and that can be made available to the entire world population (Osterholm 2005). These efforts will be costly, but only in terms of money: nothing compared to the loss of life associated with the next influenza pandemic.

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184 Treatment and Prophylaxis

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186 Treatment and Prophylaxis

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Chapter 10: Drug Profiles

Bernd Sebastian Kamps and Christian Hoffmann

Amantadine

Amantadine inhibits the replication of influenza A viruses by interfering with the uncoating of the virus inside the cell. Like rimantadine, it is an M2 inhibitor which blocks the ion channel formed by the M2 protein that spans the viral membrane (Hay 1985, Sugrue 1991). The influenza virus enters its host cell by receptor-mediated endocytosis. Thereafter, acidification of the endocytotic vesicles is required for the dissociation of the M1 protein from the ribonucleoprotein complexes. Only then are the ribonucleoprotein particles imported into the nucleus via the nuclear pores. The hydrogen ions needed for acidification pass through the M2 channel. Amantadine blocks the channel (Bui 1996).

Amantadine is effective against all influenza A subtypes that have previously caused disease in humans (H1N1, H2N2 and H3N2), but not against influenza B virus, because the protein M2 is unique to influenza A viruses. For both the prevention and treatment of influenza A, amantadine has a similar efficacy to rimantadine (Stephenson 2001, Jefferson 2004). Comparative studies indicate that adverse effects were significantly more common with amantadine than rimantadine (Jefferson 2004). Amantadine is not active against the avian influenza subtype H5N1 strains which have recently caused disease in humans (Li 2004). Besides influenza, amantadine may also be indicated in the treatment of Parkinson's disease and drug-induced extrapyramidal reactions. Moreover, it may be effective as an adjunct to interferon-based combination therapy in patients with chronic hepatitis C who have failed prior hepatitis C therapy (Lim 2005).

With daily costs of 0.50 € per day in some European countries, amantadine is by far the cheapest treatment for influenza A, compared to daily costs of 5 € for rimantadine and 7 € for oseltamivir.

The use of amantadine is associated with the rapid emergence of drug-resistant variants. Resistant isolates of influenza A are genetically stable and fully transmissible, and the pathogenic potential is comparable to that of wild-type virus isolates. In immunocompromised patients, resistant virus can be shed for prolonged periods (Boivin 2002). According to a study which assessed more than 7,000 influenza A virus samples obtained from 1994 to 2005, drug resistance against amantadine and rimantadine has increased worldwide from 0.4 % to 12.3 % (Bright 2005). Virus samples collected in 2004 from South Korea, Taiwan, Hong Kong, and China showed drug-resistance frequencies of 15 %, 23 %, 70 %, and 74 %, respectively. Some authors have suggested that the use of amantadine and rimantadine should be frankly discouraged (Jefferson 2006). Recently, 109 out of 120 (91 %) influenza A H3N2 viruses isolated from patients in the US contained an amino acid change at position 31 of the M2 protein, which confers resistance to amantadine and rimantadine. On the basis of these results, the Centre for Disease Control recommended that neither amantadine nor rimantadine be used for the treatment or prophylaxis of

influenza A in the United States for the remainder of the 2005–06 influenza season (CDC 2006).

Pharmacokinetics

Amantadine is well absorbed orally and maximum drug concentrations (C_{max}) are directly dose-related for doses of up to 200 mg/day. Doses above 200 mg/day may result in disproportional increases in C_{max} . In healthy volunteers, peak concentration were reached after 3 hours and the half-life was 17 hours (range: 10 to 25 hours). Amantadine is primarily excreted unchanged in the urine by glomerular filtration and tubular secretion.

In individuals older than 60 years, the plasma clearance of amantadine is reduced and the plasma half-life and plasma concentrations are increased. The clearance is also reduced in patients with renal insufficiency: the elimination half-life increases two to three fold or greater when creatinine clearance is less than 40 ml/min and averages eight days in patients on chronic haemodialysis. Amantadine is not removed by haemodialysis.

As the excretion rate of amantadine increases rapidly when the urine is acidic, the administration of urine acidifying drugs may increase the elimination of the drug from the body.

Toxicity

Gastrointestinal symptoms – mainly nausea but also vomiting, diarrhoea, constipation, and loss of appetite – are the major side effects. In addition, amantadine has a wide range of toxicities which may be in part attributable to the anticholinergic effects of the drug, and some reversible CNS side effects may occur during a 5-day-treatment in a substantial number of patients (van Voris 1981). As the occurrence of adverse effects is dose-related, adverse events are particularly common in the elderly and those with impaired renal function. Side effects begin within two days of the start of the drug, and usually disappear rapidly after cessation of treatment.

CNS toxicity may manifest as dizziness, nervousness, and insomnia. In a four-week prophylaxis trial, these symptoms occurred in up to 33 % of young individuals (Bryson 1980). Decreased performance on sustained attention tasks was also observed. Other CNS adverse effects include agitation, difficulty concentrating, insomnia, and lowered seizure threshold. In a direct comparison of the prophylactic use of amantadine and rimantadine, more patients receiving amantadine (13 % vs. 6% on rimantidine) withdrew from the study because of CNS side effects (Dolin 1982).

Less frequently (1-5 %) reported adverse reactions are: depression, anxiety and irritability, hallucinations, confusion, anorexia, dry mouth, constipation, ataxia, livedo reticularis, peripheral oedema, orthostatic hypotension, headache, somnolence, dream abnormality, agitation, dry nose, diarrhoea and fatigue (Symmetrel 2003).

Deaths have been reported from overdose with amantadine. The lowest reported acute lethal dose was 1 gram. In the past, some patients attempted suicide by overdosing with amantadine. As a result, it is recommended that the minimum quantity of drug is prescribed (Symmetrel 2003).

Acute toxicity may be attributable to the anticholinergic effects of amantadine. Drug overdose has therefore resulted in cardiac, respiratory, renal or central nerv-

ous system toxicity. There is no specific antidote. For more information, please check the prescribing information ([Symmetrel 2003](#)).

Efficacy

In a Cochrane review of 15 placebo-controlled trials on the prophylactic effect of amantadine, amantadine prevented 61 % of influenza cases and 25 % of cases of influenza-like illness but had no effect on asymptomatic cases ([Jefferson 2006](#)). In treatment, amantadine significantly shortened the duration of fever (by 0.99 days) but had no effect on nasal shedding of influenza A virus. The low efficacy of amantadine together with the relatively high rate of adverse events led the authors to conclude that the use of amantadine should be discouraged in seasonal and pandemic influenza ([Jefferson 2006](#)) (see also the CDC recommendation in the Introduction).

Resistance

Point mutations in the M gene lead to amino acid changes in the transmembrane region of the M2 protein and may confer high-level resistance to amantadine. The five amino acid sites known to be involved are 26, 27, 30, 31, and 34 ([Holsinger 1994](#)). The use of amantadine for treatment has been associated with the rapid emergence of resistant viruses capable of transmission, compromising its potential as a prophylaxis as well its efficacy as a treatment ([Fleming 2003](#)). The mutants are as virulent and transmissible as the wild-type virus. In an avian model, they were also genetically stable, showing no reversion to the wild-type after several passages in birds ([Bean 1989](#)). These results suggest that resistant mutants may have the potential to threaten the effective use of amantadine for the control of epidemic influenza.

Drug Interactions

Amantadine adds to the sedating effects of alcohol and other sedating drugs such as benzodiazepines, tricyclic antidepressants, dicyclomine, certain antihistamines, opiate agonists and certain antihypertensive medications. Such combinations can cause dizziness, confusion, light headedness, or fainting.

Co-administration of quinine or quinidine with amantadine has been shown to reduce the renal clearance of amantadine by about 30 % ([Gaudry 1993](#)).

Co-administration of thioridazine can worsen the tremor in elderly patients with Parkinson's disease.

Recommendations for Use

Amantadine does not completely prevent the host immune response to influenza A infection ([Sears 1987](#)) – individuals who take the drug may still develop immune responses to the natural disease or vaccination and may be protected when exposed at a later date to antigenically related viruses.

EU

In the EU, indications for influenza A treatment vary between the member states (i.e., indicated for treatment and/or prophylaxis of adults; or adults and children; or only adults and adolescents). Please check the prescribing information.

US

In the US, amantadine is indicated for the **treatment** of uncomplicated respiratory tract illness caused by influenza A virus strains. Treatment should be started as soon as possible, preferably within 24 to 48 hours after the onset of symptoms, and should be continued for 24 to 48 hours after the disappearance of clinical signs.

Amantadine is also indicated for **prophylaxis** against the signs and symptoms of influenza A virus infection when early vaccination is not feasible or when the vaccine is contraindicated or not available. Prophylactic dosing should be started in anticipation of an influenza A outbreak and before or after contact with individuals with influenza A virus respiratory tract illness.

Amantadine should be continued for at least 10 days following known exposure. When prophylaxis is started with inactivated influenza A virus vaccine, it should be administered for 2 to 4 weeks after the vaccine has been given (i.e., until protective antibody responses develop). When inactivated influenza A virus vaccine is unavailable or contraindicated, amantadine should be administered for the duration of known influenza A infection in the community because of repeated and unknown exposure.

The **daily dosage** of amantadine for adults is 200 mg; two 100 mg tablets (or four teaspoonfuls of syrup) as a once daily dose. The daily dosage may be split into one tablet of 100 mg twice a day. If central nervous system effects develop on a once daily dosage, a split dosage schedule may reduce such complaints. In persons of 65 years of age or older, the daily dosage of amantadine is 100 mg. Low-dose amantadine (100 mg/day) can reduce toxicity and may maintain the prophylactic efficacy seen with 200 mg/day (Sears 1987). In an experimental challenge study on 78 subjects, using doses of 50 mg, 100 mg or 200 mg/day, there was no significant difference between the groups in influenza illness or viral shedding (Reuman 1989).

In elderly institutionalised patients, individualised dosing of amantadine, based upon a patient's creatinine clearance, seems to be effective while reducing adverse reactions (Kolbe 2003).

In **paediatric patients**, lower total daily doses should be calculated on the basis of 4.4 to 8.8 mg/kg/day (2 to 4 mg/lb/day). However, given the relatively low efficacy of amantadine and the high risk of occurrence of gastrointestinal and CNS adverse effects, the authors do not recommend the administration of amantadine in children.

Warnings

Amantadine is contraindicated in severe renal impairment and patients with epilepsy. In addition, it should be used cautiously in elderly patients (impaired renal function?).

Amantadine may cause mydriasis and should therefore not be given to patients with untreated closed-angle glaucoma.

The safety of amantadine in pregnant women has not been established.

The dose of amantadine may need careful adjustment in patients with congestive heart failure, peripheral oedema, or orthostatic hypotension. Care should be exercised when administering amantadine to patients with a history of recurrent eczematoid rash, or to patients with psychosis or severe psychoneurosis not controlled by chemotherapeutic agents (Symmetrel 2003).

Summary

Amantadine is available as 100 mg tablets or capsules and as a syrup containing 50 mg/5ml.

Drug class: M2-inhibitor.

Indications: treatment and prevention of Influenza A.

Dosage: 200 mg qd both for treatment and prophylaxis. For prophylaxis, amantadine should be started as soon as possible after exposure and continued for at least 10 days.

Special Dosage: persons with reduced kidney function and elderly persons may need lower doses (or less frequent doses).

Pharmacokinetics: good absorption with peak concentration after 3 hours and a half-life of 17 hours. Excreted unchanged in the urine by glomerular filtration and tubular secretion. Reduced clearance in individuals > 60 years and in patients with renal insufficiency: half-life is increased when creatinine clearance is less than 40 ml/min. Amantadine is not removed by haemodialysis.

Contraindications: psychosis. Patients with insufficiently treated epileptic episodes.

Interactions: central nervous system stimulants; quinine and quinidine; thioridazine.

Side effects: gastrointestinal and CNS symptoms.

Comments/Warnings: no well-controlled studies have been done in pregnant women to evaluate the safety of amantadine. Amantadine should not be prescribed to pregnant women.

Amantadine is excreted in breast milk in low concentrations. Although no information is available on the effects in infants, the manufacturer recommends that amantadine be used cautiously in nursing mothers.

Patients receiving amantadine who note central nervous system effects or blurring of vision should be cautioned against driving or working in situations where alertness and adequate motor co-ordination are important.

Store amantadine at room temperature between 15 and 30°C (59 and 86°F).

Internet sources:

USA: <http://influenzareport.com/link.php?id=6>

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Oseltamivir

Introduction

Oseltamivir is a potent and selective inhibitor of the neuraminidase enzyme of the influenza viruses A and B. The neuraminidase enzyme is responsible for cleaving sialic acid residues on newly formed virions and plays an essential role in the release and spread of progeny virions. When exposed to oseltamivir, the influenza virions aggregate on the surface of the host cell, thereby limiting the extent of infection within the mucosal secretions (McNicholl 2001) and reducing viral infectivity.

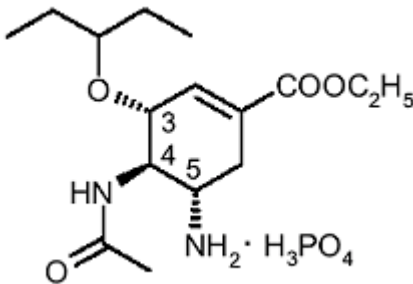
Oseltamivir is indicated in the prophylaxis of influenza and for the treatment of uncomplicated acute illness due to influenza in patients 1 year and older who have been symptomatic for no more than 2 days. H5N1 strains are generally sensitive against oseltamivir, but there are no data on its clinical efficacy.

Clinical studies have shown that neuraminidase inhibitors can decrease the duration of influenza-related symptoms if initiated within 48 hours of onset. Clinical efficacy is about 60-70 % and, for treatment started within 48 hours, symptoms such as myalgias, fever, and headache were reduced by approximately 0.7-1.5 days (McNicholl 2001). Treatment is more effective if initiated within 30 hours of symptom onset in febrile individuals. Treatment with oseltamivir does not seem to adversely affect the primary in vivo cellular immune responses to influenza virus infection (Burger 2000).

Oseltamivir is generally well-tolerated with the only clinically important side effect being mild gastrointestinal upset (Doucette 2001). Recently, the drug has been linked to a number of cases of psychological disorders and two teenage suicides in Japan. However, there is currently no evidence of a causal relationship between oseltamivir intake and suicide.

Structure

Oseltamivir is an ethyl ester prodrug which requires ester hydrolysis to be converted to the active form, oseltamivir carboxylate [3R,4R,5S]-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate. The discovery of oseltamivir was possible through rational drug design utilising available x-ray crystal structures of sialic acid analogues bound to the active site of the influenza virus neuraminidase (Lew 2000). Oseltamivir was developed through modifications to the sialic acid analogue framework (including the addition of a lipophilic side chain) that allow the drug to be used orally (Kim 1998). The structural formula is as follows:



During its early development, oseltamivir and its active metabolite were known as GS4104 and Ro 64-0796, and GS4071 and Ro 64-0802, respectively.

Pharmacokinetics

Following oral administration, oseltamivir is readily absorbed from the gastrointestinal tract. After conversion to the active metabolite oseltamivir carboxylate in the liver, it distributes throughout the body, including the upper and lower respiratory tract (Doucette 2001). The absolute bioavailability of the active metabolite from orally administered oseltamivir is 80%. The active metabolite is detectable in plasma within 30 minutes and reaches maximum concentrations after 3 to 4 hours. Once peak plasma concentrations have been attained, the concentration of the active metabolite declines with an apparent half-life of 6 to 10 hours (He 1999).

The terminal plasma elimination half-life is 1.8 h in healthy adults. In patients with renal impairment, metabolite clearance decreases linearly with creatinine clearance, and averages 23 h after oral administration in individuals with a creatinine clearance < 30 ml/min (Doucette 2001). A dosage reduction to 75 mg once daily is recommended for patients with a creatinine clearance < 30 ml/min (1.8 l/h) (He 1999).

Plasma protein binding is 3%. The drug and the active metabolite are excreted by glomerular filtration and active tubular secretion without further metabolism (Hill 2001). Neither compound interacts with cytochrome P450 mixed-function oxidases or glucuronosyltransferases (He 1999). Thus, the potential is low for drug-drug interactions, which appear to be limited to those arising from competitive inhibition of excretion by the renal tubular epithelial cell anionic transporter. Probenecid blocks the renal secretion of oseltamivir, more than doubling systemic exposure oseltamivir carboxylate (Hill 2002). This competition is unlikely to be clinically

relevant, but there has been speculation about using probenecid to “stretch” oseltamivir stocks in situations of pandemic shortage (Butler 2005).

The metabolism of oseltamivir is not compromised in hepatically impaired patients and no dose adjustment is required (Snell 2005).

In elderly individuals, exposure to the active metabolite at steady state is approximately 25 % higher compared with young individuals; however, no dosage adjustment is necessary (He 1999).

Young children 1 to 12 years of age clear the active metabolite oseltamivir carboxylate at a faster rate than older children and adults, resulting in lower exposure. Increasing the dose to 2 mg/kg twice daily resulted in drug exposures comparable to the standard 1 mg/kg twice daily dose used in adults (Oo 2001). Infants as young as 1 year old can metabolise and excrete oseltamivir efficiently (Oo, 2003). In younger children, use of oseltamivir is contraindicated (see Toxicity).

Toxicity

The most frequent side effects are nausea and vomiting which are generally of a mild to moderate degree and usually occur within the first 2 days of treatment.

The following adverse reactions have been identified during post-marketing use of oseltamivir. In many cases, it is not possible to reliably estimate their frequency or establish a cause relationship to oseltamivir exposure:

- Rash, swelling of the face or tongue, toxic epidermal necrolysis
- Hepatitis, abnormal liver function tests
- Arrhythmias
- Seizures, confusion
- Aggravation of diabetes

Oseltamivir use does not appear to be associated with an increased risk of skin reactions (Nordstrom 2004); however, anecdotal reports describe isolated skin reactions, i.e. the case of generalised rash after prophylactic use of oseltamivir and zanamivir in two patients with hepatoma associated with liver cirrhosis (Kaji 2005). After a comprehensive review of the available data, the FDA has recently required serious skin/hypersensitivity reactions be added to the oseltamivir product label. Patients should be cautioned to stop taking oseltamivir and contact their health care providers if they develop a severe rash or allergic symptoms (FDA 2005).

The use of oseltamivir in infants younger than 1 year is not recommended as studies on juvenile rats revealed potential toxicity of oseltamivir for this age group. Moreover, high drug levels were found in the brains of 7-day-old rats which were exposed to a single dose of 1,000 mg/kg oseltamivir phosphate (about 250 times the recommended dose in children). Further studies showed the levels of oseltamivir phosphate in the brain to be approximately 1,500 times those seen in adult animals. The clinical significance of these preclinical data for human infants is uncertain. However, given the uncertainty in predicting the exposure in infants with immature blood-brain barriers, it is recommended that oseltamivir not be administered to children younger than 1 year, the age at which the human blood-brain barrier is generally recognised to be fully developed (Dear Doctor-Letter, <http://InfluenzaReport.com/link.php?id=2>).

Oseltamivir is a pregnancy category C drug, as there are insufficient human data upon which to base a risk evaluation of oseltamivir to the pregnant woman or developing foetus.

In lactating rats, oseltamivir is excreted in the milk, but oseltamivir has not been studied in nursing mothers and it is not known, if oseltamivir is excreted in human milk.

After reports of psychological disorders in patients treated with oseltamivir, Japanese authorities have amended the patient information to list psychiatric effects, such as delusions, in the list of side effects.

Efficacy

Treatment

Oseltamivir, 75 mg bid for 5 days, administered to otherwise healthy adults with naturally acquired febrile influenza when started within 36 hours of the onset of symptoms, reduced the duration of the disease by up to 1.5 days and the severity of illness by up to 38 % (Treanor 2000). Earlier initiation of therapy was associated with a faster resolution: initiation of therapy within the first 12 h after fever onset reduced the total median illness duration 3 days more than intervention at 48 h. In addition, the earlier administration of oseltamivir reduced the duration of fever, severity of symptoms and the times to return to baseline activity (Aoki 2003).

Body temperature exceeding 39°C was an indicator of a longer duration of fever (Kawai 2005). The effect of oseltamivir may be apparent within 24 h of the start of treatment (Nichson 2000). A meta-analysis of 10 placebo-controlled, double-blind trials suggests that oseltamivir treatment of influenza illness reduces lower respiratory tract complications, use of antibacterials, and hospitalisation in both healthy and “at-risk” adults (Kaiser 2003).

The efficacy and safety of oseltamivir in patients with chronic respiratory diseases (chronic bronchitis, obstructive emphysema, bronchial asthma or bronchiectasis) or chronic cardiac disease has not been well defined. In one small randomised trial oseltamivir significantly reduced the incidence of complications (11 % vs. 45 %) and antibiotic use (37 % vs. 69 %) in the treatment group compared with the control group (Lin 2006). The cost of treating influenza and its complications was comparable between the two groups.

Oseltamivir treatment may be less effective for influenza B than for influenza A (for efficacy against H5N1 strains, see below).

A cost-utility decision model, including epidemiological data and data from clinical trials of antiviral drugs, concluded that for unvaccinated or high-risk vaccinated patients, empirical oseltamivir treatment seems to be cost-effective during the influenza season, while for other patients, treatment initiation should await the results of rapid diagnostic testing (Rothberg 2003).

Prophylaxis

When used in experimentally infected individuals, prophylactic use of oseltamivir resulted in a reduced number of infections (8/21 in the placebo group and 8/12 in the oseltamivir group) and infection-related respiratory illness (4/12 vs. 0/21; $p=0.16$; efficacy, 61 %) (Hayden 1999a). These findings were confirmed by a clinical trial in 1,559 healthy, non-immunised adults aged 18 to 65 years, who received either

oral oseltamivir (75 mg or 150 mg daily) or placebo for six weeks during a peak period of local influenza activity (Hayden 1999b). The risk of influenza among subjects assigned to oseltamivir (1.2 %) was lower than that among subjects assigned to placebo (4.8 %), yielding a protective efficacy of oseltamivir of 74 per cent (Hayden 1999a). A meta-analysis of seven prevention trials showed that prophylaxis with oseltamivir reduced the risk of developing influenza by 70-90 % (Cooper 2003).

When administered prophylactically to household contacts of an influenza index case (IC), once daily for 7 days within 48 hours of the onset of symptoms in the IC, oseltamivir had an overall protective efficacy against clinical influenza of 89 % (Welliver 2001). In a randomised trial, 12.6 % (26/206) laboratory-confirmed clinical influenza episodes occurred in the placebo group vs. 1.4 % (3/209) in the oseltamivir group. In another randomised study, efficacy of post-exposure prophylaxis (PEP) and treatment of ill index cases was determined: household contacts of index cases presenting with an influenza-like illness (defined by temperature $\geq 37.8^{\circ}\text{C}$ plus cough and/or coryza) were randomised to receive PEP with oseltamivir for 10 days or treatment at the time of developing illness during the postexposure period. All index cases received oseltamivir treatment for 5 days (Hayden 2004). PEP was found to have a protective efficacy of 68 % against proven influenza, compared with treatment of index cases alone: 13 % (33/258) episodes of influenza illness in the placebo group vs. 4 % (10/244) in the oseltamivir group ($p=0.017$).

A cost-effectiveness analysis based on a decision analytic model from a government-payer perspective calculated that the use of oseltamivir post-exposure prophylaxis is more cost-effective than amantadine prophylaxis or no prophylaxis (Risebrough 2005). Another recent meta-analysis, however, found a relatively low efficacy of oseltamivir (Jefferson 2006), leading the authors to conclude that oseltamivir should not be used in seasonal influenza control and should only be used in a serious epidemic and pandemic alongside other public health measures.

Selected Patient Populations

A double-blind, placebo-controlled study investigated the efficacy of once-daily oral oseltamivir for 6 weeks as a prophylaxis against laboratory-confirmed clinical influenza in 548 **frail older people** (mean age 81 years, > 80 % vaccinated) living in homes for seniors (Peters 2001). Compared with placebo, oseltamivir resulted in a 92 % reduction in the incidence of laboratory-confirmed clinical influenza ($1/276 = 0.4\%$ versus $12/272 = 4.4\%$). Oseltamivir also significantly reduced the incidence of secondary complications (Peters 2001).

Children: oral oseltamivir treatment in **paediatric patients** reduced the median duration of illness by 36 h and also cough, coryza and duration of fever. In addition, new diagnoses of otitis media were reduced by 44 % and the incidence of physician-prescribed antibiotics was lower (Whitley 2001). In a recent study, oseltamivir was well-tolerated among asthmatic children and might help to reduce symptom duration and improve lung function. Patients treated with oseltamivir also experienced fewer asthma exacerbations (51 % versus 68 %) (Johnston 2005).

The efficacy of oseltamivir in the treatment of subjects with **chronic cardiac disease and/or respiratory disease** has not been established. No information is available regarding treatment of influenza in patients with any medical condition suffi-

ciently severe or unstable to be considered at imminent risk of requiring hospitalisation. In patients who have undergone **bone-marrow transplantation**, oseltamivir might be an option during the first 6 months after transplantation when preventive vaccination strategies are precluded due to poor immunogenicity of the vaccine during this period (Machado 2004).

Efficacy against Avian Influenza H5N1

In vitro studies have demonstrated a potent antiviral activity against all strains of influenza A and B including the avian H5N1 and H9N2 strains implicated in the human cases in Hong Kong (Leneva 2000). A review of H5N1 influenza cases, led by the WHO, suggested that viral shedding and infectivity of index cases could be reduced (Writing Committee of the WHO 2005). However, the clinical benefit of oseltamivir in avian influenza infections in humans remains poorly defined. Recent observations suggest that in some patients with H5N1 virus infection, treatment with the recommended dose of oseltamivir incompletely suppresses viral replication, providing opportunities for drug resistance to develop (de Jong 2005). Whether oseltamivir needs to be used in higher doses, or over longer periods of time than currently recommended, is still subject to debate. Another open question is the initiation of treatment late in the course of illness, when there is evidence of ongoing viral replication. There is some very limited evidence that even late treatment initiation reduces viral load to undetectable levels and may have contributed to the survival of some patients (de Jong 2005). These findings would be consistent with studies in mice inoculated with H5N1. While a 5-day regimen at 10 mg/kg/day protected 50 % of mice, 8-day regimens demonstrated an 80 % survival rate (Yen 2005b). In another study, treatment with oseltamivir improved survival in mice from 0 % to 75 %, even when therapy was delayed for up to 5 days after infection with influenza virus (McCullers 2004).

Higher doses of oseltamivir in humans could be safe. Data from dose ranging studies show that 5 day courses of 150 mg twice daily for treatment and 6 week courses of 75 mg twice daily for prophylaxis were as well tolerated as the approved dose regimens (Ward 2005).

Efficacy against the 1918 Influenza Strain

Recombinant viruses possessing the 1918 NA or both the 1918 HA and 1918 NA were inhibited effectively in both tissue culture and mice by oseltamivir, suggesting that oseltamivir would be effective against a re-emergent 1918 or 1918-like virus (Tumpey 2002).

Resistance

In vitro, the NA mutations E119V, R292K, H274Y, and R152K are associated with resistance to oseltamivir (McKimm-Breschkin 2003). Viral strains containing the R292K mutation did not replicate as well as the wild-type virus in culture and were 10,000-fold less infectious than the wild-type virus in a mouse model (Tai 1998). Likewise, the H274Y mutation reduced the replicative ability in cell culture by up to 3 logs (Ives 2002), required a 100-fold-higher dose for infection of donor ferrets, and was transmitted more slowly than was the wild type (Herlocher 2004).

It has been suggested that if mutations compromise viral fitness, they might be without clinical significance. The recently published cases of high-level resistance

to oseltamivir in an H5N1 strain shed some doubt on this hypothesis (Le 2005, de Jong 2005). In this case, treatment with the recommended dose of oseltamivir, although started one day after the onset of symptoms, did not suppress viral replication efficiently and eventually led to the development of a drug-resistant strain. The cause for this – overwhelming viral replication or altered pharmacokinetics in severely ill patients – is unclear.

Whereas the incidence of development of resistant strains of seasonal H1N1 and H3N2 influenza has been low among adults and adolescents (0.3 %), paediatric studies have demonstrated higher rates. One study found neuraminidase mutations in viruses from 9/50 patients (18 %), six of whom had mutations at position 292 and two at position 119 (Kiso 2004). As children can be a source of viral transmission, even after 5 days of treatment with oseltamivir, the implications of these findings need to be investigated.

Cross-resistance between oseltamivir-resistant influenza mutants and zanamivir-resistant influenza mutants has been observed in vitro. Two of the three oseltamivir-induced mutations (E119V, H274Y and R292K) in the neuraminidase from clinical isolates occur at the same amino acid residues as two of the three mutations (E119G/A/D, R152K and R292K) observed in zanamivir-resistant virus (Tamiflu 2005).

Drug Interactions

Information derived from pharmacology and pharmacokinetic studies suggests that clinically significant drug interactions are unlikely (Tamiflu 2005). Neither oseltamivir nor oseltamivir carboxylate is a substrate for, or inhibitor of, cytochrome P450 isoforms.

Recommendations for Use

EU

Oseltamivir (Tamiflu[®]) has been approved centrally within the European Union. Treatment indications and dosages correspond to the US marketing authorisation.

US

In the US, oseltamivir is indicated for the treatment of uncomplicated acute illness due to influenza infection in patients aged 1 year and older who have been symptomatic for no more than 2 days. In addition, oseltamivir is indicated for the prophylaxis of influenza in patients aged 1 year and older.

The standard dosage for **treatment** of patients 13 years of age and older is 75 mg bid for 5 days. Paediatric patients or adults who cannot swallow capsules, receive oseltamivir 30, 45 and 60 mg oral suspension twice daily. Recommended dose:

Body Weight	Recommended Dose for 5 Days
≤ 15 kg (≤ 33 lb)	30 mg twice daily
> 15 kg to 23 kg (> 33 lb to 51 lb)	45 mg twice daily
> 23 kg to 40 kg (> 51 lb to 88 lb)	60 mg twice daily
> 40 kg (> 88 lb)	75 mg twice daily

A 75 mg capsule may be a viable formulation in children (e.g. over 8 years of age) who are able to swallow solid dosage forms.

For **prophylaxis**, the recommended dose is 75 mg once daily for at least 7 days. The recommended oral dose of oseltamivir suspension for paediatric patients aged 1 year and older following contact with an infected individual:

Body Weight	Recommended Dose for 7 Days
≤ 15 kg (≤ 33 lb)	30 mg once daily
> 15 kg to 23 kg (> 33 lb to 51 lb)	45 mg once daily
> 23 kg to 40 kg (> 51 lb to 88 lb)	60 mg once daily
> 40 kg (> 88 lb)	75 mg once daily

Summary

Oseltamivir is a selective neuraminidase inhibitor. Treatment must start within 48 hours after the onset of symptoms, but is most effective if initiated as soon as possible (< 24 hours). The drug is generally well-tolerated.

Oseltamivir is not a substitute for early vaccination on an annual basis, as recommended by the national authorities.

Efficacy, optimal dosage and duration of treatment in H5N1 infection has still to be defined.

Trade name: Tamiflu™

75 mg capsules (blister packages of 10).

Powder for oral suspension, to be constituted with water (12 mg/ml; available in glass bottles containing 25 ml of suspension).

Drug class: neuraminidase inhibitor.

Manufacturer: Hoffmann-La Roche.

Indications: uncomplicated acute illness due to influenza infection in patients aged 1 year and older who have been symptomatic for no more than 2 days.

Prophylaxis of influenza in patients older than 1 year.

Standard Dosage for Treatment: 75 mg bid for 5 days.

Paediatric patients or adults who cannot swallow, receive the oral suspension. Recommended dose: see above.

Standard Dosage for Prophylaxis: 75 mg once daily for at least 7 days following contact with an infected individual.

Paediatric patients or adults who cannot swallow, receive the oral suspension. Recommended dose: see above.

Special Dosage: patients with a serum creatinine clearance between 10 and 30 ml/min are treated with 75 mg once daily for 5 days; the prophylactic dose is 75 mg every other day or 30 mg oral suspension every day. No recommended dosing regimens are available for patients undergoing routine haemodialysis and continuous peritoneal dialysis treatment with end-stage renal disease.

Pharmacokinetics: oseltamivir is readily absorbed from the gastrointestinal tract following oral administration and is extensively converted to oseltamivir carboxylate. Oseltamivir carboxylate is eliminated in the urine with a half-life of 6 to 10 hours.

Contraindications: oseltamivir is not indicated for the treatment of influenza in paediatric patients younger than 1 year.

Oseltamivir should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus (Pregnancy Category C).

Interactions: significant drug interactions are unlikely.

Side effects: the most frequent side effects are nausea and vomiting which are generally mild to moderate in degree and usually occur on the first 2 days of treatment.

Comments/Warnings: patients should be instructed to begin treatment with oseltamivir as soon as possible after the first appearance of flu symptoms. Similarly, prevention should begin as soon as possible following exposure.

Transient gastrointestinal disturbance may be reduced by taking oseltamivir after a light snack.

No dose adjustment is required for geriatric patients.

Co-administration with food has no significant effect on the peak plasma concentration and the AUC.

Store capsules at 25°C (77° F); excursions permitted to 15° to 30° C (59° to 86° F).

It is recommended that the oral suspension be constituted by the pharmacist prior to dispensing to the patient (see the product information on the Internet).

Store constituted suspension under refrigeration at 2° to 8° C (36° to 46° F). Do not freeze.

Oseltamivir is not a substitute for a flu vaccination. Patients should continue receiving an annual flu vaccination according to the national guidelines on immunisation practices.

Internet sources:

EU: <http://influenzareport.com/link.php?id=14>

USA: <http://influenzareport.com/link.php?id=1>

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204 Drug Profiles

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Rimantadine

Introduction

Rimantadine is an M2 ion channel inhibitor which specifically inhibits the replication of influenza A viruses by interfering with the uncoating process of the virus. M2 inhibitors block the ion channel formed by the M2 protein that spans the viral membrane (Hay 1985, Sugrue 1991). The influenza virus enters its host cell by receptor-mediated endocytosis. Thereafter, acidification of the endocytotic vesicles is required for the dissociation of the M1 protein from the ribonucleoprotein complexes. Only then are the ribonucleoprotein particles imported into the nucleus via the nuclear pores. The hydrogen ions needed for acidification pass through the M2 channel. Rimantadine blocks the channel (Bui 1996).

The drug is effective against all influenza A subtypes that have previously caused disease in humans (H1N1, H2N2 and H3N2), but not against influenza B virus, because the M2 protein is unique to influenza A viruses. Rimantadine is not active against the avian flu subtype H5N1 strains that have recently caused disease in humans (Li 2004).

For both the prevention and treatment of influenza A, rimantadine has a comparable efficacy to amantadine but a lower potential for causing adverse effects (Stephenson 2001, Jefferson 2004).

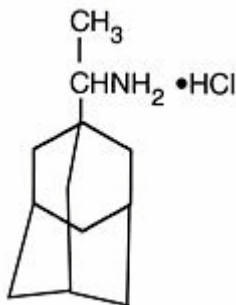
The development of neutralising antibodies to influenza strains seems not to be affected by rimantadine. However, the presence of IgA in nasal secretions was significantly diminished in one study (Clover 1991).

A recently published study revealed an alarming increase in the incidence of amantadine-resistant and rimantadine-resistant H3N2 influenza A viruses over the past decade. In a recently published study, which assessed more than 7,000 influenza A viruses obtained worldwide from 1994 to 2005, drug resistance against amantadine and rimantadine increased from 0.4 % to 12.3 % (Bright 2005). Viruses collected in 2004 from South Korea, Taiwan, Hong Kong, and China show drug-resistance frequencies of 15 %, 23 %, 70 %, and 74 %, respectively. Some authors have suggested that the use of amantadine and rimantadine should be discouraged (Jefferson 2006). Recently, 109 out of 120 (91 %) of influenza A H3N2 viruses isolated from patients in the US contained an amino acid change at position 31 of the M2 protein, which confers resistance to amantadine and rimantadine. On the basis of these results, the Centre for Disease Control recommended that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza A in the United States for the remainder of the 2005–06 influenza season (CDC 2006).

In most countries, rimantadine is not available.

Structure

Chemically, rimantadine hydrochloride is alpha-methyltricyclo-[3.3.1.1/3.7]decane-1-methanamine hydrochloride, with a molecular weight of 215.77 and the following structural formula:



Pharmacokinetics

In healthy adults, peak plasma concentrations are reached 6 hours after oral administration. The single dose elimination half-life is about 30 hours in both adults (Hayden 1985) and children (Anderson 1987). Following oral administration, rimantadine is extensively metabolised in the liver and less than 25 % of the dose is excreted unchanged in the urine. In elderly people, the elimination is prolonged, with average AUC values and peak concentrations being 20 to 30 % higher than in healthy adults.

In chronic liver disease, rimantadine pharmacokinetics are not appreciably altered (Wills 1987); however, in patients with severe hepatic insufficiency, the AUC and the elimination half-life time are increased.

Renal insufficiency results in increased plasma concentrations of rimantadine metabolites. Haemodialysis does not remove rimantadine. Rimantadine dosage may therefore need to be reduced in patients with end-stage renal disease. Supplemental doses on dialysis days are not required (Capparelli 1988).

Toxicity

Gastrointestinal symptoms are the most frequent adverse events associated with rimantadine. Other side effects noted during clinical trials (all < 3 %) included nausea, vomiting, anorexia, and dry mouth, as well as CNS symptoms (insomnia, dizziness, nervousness). However, a study on the safety and efficacy of prophylactic long-term use in nursing homes showed no statistically significant differences in the frequencies of gastrointestinal or central nervous system symptoms between treatment and placebo groups (Monto 1995).

Less frequent adverse events (0.3 to 1 %) were diarrhoea, dyspepsia, impairment of concentration, ataxia, somnolence, agitation, depression, rash, tinnitus, and dyspnoea.

Rarely, seizures may develop in patients with a history of seizures, who are not receiving anticonvulsant medication. In these cases, rimantadine should be discontinued.

Generally, symptoms resolve rapidly after discontinuation of treatment.

The safety and pharmacokinetics of rimantadine in renal and hepatic insufficiency have only been evaluated after single-dose administration. Because of the potential for accumulation of rimantadine and its metabolites in plasma, caution should be exercised when treating patients with renal or hepatic insufficiency.

No well-controlled studies have been done in pregnant women to evaluate the safety of amantadine. We thus recommend that rimantadine is not prescribed for pregnant women. Likewise, rimantadine should not be administered to nursing mothers because of the adverse effects noted in the offspring of rats treated with rimantadine during the nursing period.

Comparative studies indicate that rimantadine is better tolerated than amantadine at equivalent doses (Jefferson 2004). In a direct comparison of prophylactic use of amantadine and rimantadine, more patients on amantadine (13 %) than recipients of rimantadine (6 %) withdrew from the study because of central nervous system side effects (Dolin 1982).

Efficacy

Rimantadine is not active against the avian flu subtype H5N1 strains that have recently caused disease in humans (Li 2004). Rimantadine may be effective for both the prevention and treatment of influenza A infection in “classic” human strains (H1N1, H2N2 and H3N2). The efficacy of rimantadine is comparable to amantadine. In a Cochrane review of 3 placebo-controlled trials on the prophylactic effect of rimantadine, however, rimantadine had only moderate effects on influenza cases and influenza-like illnesses (Jefferson 2006). In treatment, rimantadine significantly shortened the duration of fever but had no or at best moderate effect on nasal shedding of influenza A viruses. The low efficacy of rimantadine together with the relatively high rate of adverse events led the authors to conclude that the use of both M2 ion channel-blocking drugs, rimantadine and amantadine, should be discouraged in seasonal and pandemic influenza (Jefferson 2006) (see also the CDC recommendation in the Introduction).

Treatment

In early trials involving patients with uncomplicated influenza A H3N2 subtype virus infection, rimantadine treatment (200 mg/day for 5 days) was associated with significant reductions in nasal secretion viral titres, maximum temperature, time until defervescence (mean, 37 h shorter), and systemic symptoms compared with placebo (Hayden 1986). Rimantadine seems to be relatively safe even among vaccinated elderly individuals living in nursing homes (Monto 1995). In this population, a dosage reduction to 100 mg/day is recommended. In experimentally infected adults, rimantadine had no effect on nasal patency, mucociliary clearance, nasal signs, or on symptoms and signs of otologic complications (Doyle 1998).

Prophylaxis

Efficacy rates reported from prophylaxis trials vary widely. A review of clinical studies found that rimantadine was 64 % efficacious in prevention, and significantly

shortened the duration of fever by 1.27 days (Demicheli 2000). Rimantadine may also be effective in children (Clover 1986, Crawford 1988).

Resistance

Point mutations in the M gene leading to amino acid changes in the M2 protein may lead to high-level resistance to rimantadine. The mutants are as virulent and have been shown to be as transmissible as wild-type virus and to cause a typical influenza illness. Such strains may develop in up to one third of treated patients, although in immunocompromised individuals, the percentage may be even higher (Englund 1998). Drug-resistant influenza A virus (H3N2) can be recovered from rimantadine-treated children and adults as early as 2 days after starting treatment (Hayden 1991).

Transmissibility is an important aspect when using rimantadine. An early study demonstrated failure of prevention of influenza infection due to apparent transmission of drug-resistant viral strains. The study concluded that rimantadine was ineffective in protecting household members from influenza A infection (Hayden 1989).

Avian influenza virus subtype H5N1, which has been associated with the human disease in East Asia between late 2003 and early 2004, is resistant to rimantadine (asparagine residue at position 31 of the M2 protein) (Li 2004).

Over the last decade, drug resistance to amantadine and rimantadine has increased from 0.4 % to 12.3 % (Bright 2005).

Drug Interactions

No clinically substantial interactions between rimantadine and other drugs have been identified. Cimetidine seems to reduce rimantadine clearance by 18 % (Holazo 1989). Acetaminophen reduces the peak concentration and AUC values of rimantadine by 11 %. Aspirin reduces the peak plasma concentrations and the AUC of rimantadine by approximately 10 %.

Recommendations for Use

In the EU, medicinal products containing rimantadine have been approved nationally (for additional information, please check the prescribing information).

In the US, rimantadine is licensed for prophylaxis in adults and children. For treatment, rimantadine is licensed for adults only. Rimantadine (Flumadine[®]) is available as 100 mg film-coated tablets and as syrup for oral administration.

Adults

In the US, the recommended dose for both **prophylaxis** and **treatment** is 100 mg bid.

A dose reduction to 100 mg daily is recommended in patients with

- severe hepatic dysfunction
- renal failure ($\text{CrCl} \leq 10 \text{ ml/min}$)
- elderly nursing home patients (Patriarca 1984, Monto 1995).

Patients with any degree of renal insufficiency should be closely monitored, with dosage adjustments being made as necessary.

For treatment, rimantadine should be initiated within 48 hours after the onset of signs and symptoms of influenza A infection. Therapy should be continued for approximately seven days from the initial onset of symptoms.

Children

In the US, rimantadine is licensed for prophylactic use only. Children less than 10 years of age should receive 5 mg/kg but not exceeding 150 mg. Children 10 years of age or older receive the adult dose.

Warnings

Rimantadine should be used with caution in patients with epilepsy.

Summary

Trade name: Flumadine®

Drug class: M2 inhibitor

Indications: prophylaxis (adults and children) and treatment (adults only) of influenza A infection. Treatment must be initiated within 48 hours after the onset of symptoms.

Standard Dosage for Treatment: 100 mg bid.

A dose reduction to 100 mg daily is recommended in patients with severe hepatic dysfunction, renal failure ($\text{CrCl} \leq 10 \text{ ml/min}$) and in elderly nursing home patients.

Standard Dosage for Prophylaxis: 100 mg bid.

A dose reduction to 100 mg daily is recommended in patients with severe hepatic dysfunction, renal failure ($\text{CrCl} \leq 10 \text{ ml/min}$) and in elderly nursing home patients. Children less than 10 years of age should receive 5 mg/kg but not exceeding 150 mg. Children 10 years of age or older receive the adult dose.

Pharmacokinetics: peak plasma concentration is reached 6 hours after oral administration. The elimination half-life is 30 hours. Prolonged elimination in elderly people. Extensive metabolism in the liver – less than 25 % is excreted unchanged in the urine. Increased plasma concentration in patients with severe hepatic and renal insufficiency.

Interactions: no significant interactions.

Side effects: gastrointestinal symptoms.

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212 Drug Profiles

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Zanamivir

Introduction

Zanamivir is an orally inhaled powder currently approved in 19 countries for the treatment of, and in two for the prophylaxis of influenza A and B. Zanamivir is a competitive inhibitor of the neuraminidase glycoprotein, which is essential in the infective cycle of influenza viruses. It closely mimics sialic acid, the natural substrate of the neuraminidase (Varghese 1992, Varghese 1995).

Zanamivir is administered via inhalation, resulting in direct delivery to the respiratory tract, where the concentration has been calculated to be more than 1,000 times as high as the IC₅₀ for neuraminidase. The inhibitory effect starts within 10 seconds.

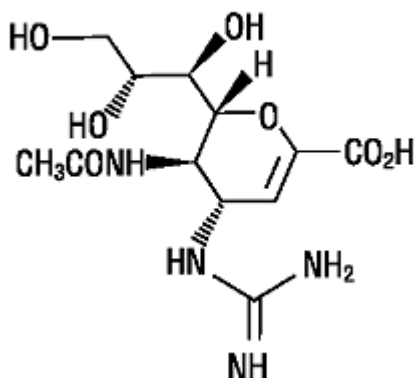
When systemic involvement of influenza infection is suspected – as has recently been suggested by some reports on avian H5N1 influenza in humans (de Jong 2005) – zanamivir might not be the suitable drug.

Over the last few years, a number of events have resulted in changes to the zanamivir prescribing information which now contains warnings of bronchospasm, dyspnoea, rash, urticaria and allergic type reactions, including facial and oropharyngeal oedema. However, apart from these rare episodes, the drug has a good safety profile if begun early (Hayden 1997).

Co-administration of orally inhaled zanamivir with inactivated trivalent influenza vaccine does not seem to adversely affect the production of antihaemagglutinin antibodies (Webster 1999); a protective antibody response develops within 12 days (Cox 2001).

Structure

The chemical name of zanamivir is 5-(acetylamino)-4-[(aminoiminomethyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid. It has the following structural formula:



Pharmacokinetics

Data on orally inhaled zanamivir indicate that 10-20 % of the active compound reaches the lungs. The rest is deposited in the oropharynx and approximately 4 % to 17 % of the inhaled dose is systemically absorbed. The peak serum concentrations are reached within 1 to 2 hours following a 10 mg dose. Plasma protein binding is limited (< 10 %). Zanamivir is excreted unchanged in the urine with the excretion of a single dose completed within 24 hours (Cass 1999b). The serum half-life of zanamivir after administration by oral inhalation ranges from 2.5 to 5.1 hours.

Studies have demonstrated that intravenously administered zanamivir is distributed to the respiratory mucosa and is protective against infection and illness following experimental human influenza A virus inoculation (Calfee 1999).

Toxicity

Zanamivir has a good safety profile and the overall risk of occurrence of any respiratory event is low (Loughlin 2002). Results from in vitro and in vivo animal studies suggest that zanamivir has low acute toxicity and no significant systemic toxicity or respiratory tract irritancy at plasma exposures more than 100-fold higher than those anticipated following clinical use (Freund 1999).

Recommended dosages of zanamivir usually do not adversely affect pulmonary function in patients with respiratory disorders. However, in some patients, bronchospasm and a decline in lung function (FEV₁ or peak expiratory flow) have been reported after usage of zanamivir. In most cases, these patients had underlying pulmonary conditions such as asthma or chronic obstructive pulmonary disease. Because of the risk of serious adverse events, zanamivir is not generally recommended for the treatment of patients with underlying airways disease. Zanamivir should also be discontinued in patients who develop bronchospasm or who have a decline in respiratory function. If symptoms are severe, immediate treatment and hospitalisation may be required.

Allergic reactions, including oropharyngeal oedema and serious skin rashes may rarely occur during treatment with zanamivir. In these cases, the drug should be stopped and appropriate treatment instituted.

The frequency of other side effects has been reported to be roughly identical in both treatment and placebo groups: diarrhoea, nausea, dizziness, headaches, less frequently malaise, abdominal pain, and urticaria occurred at similar frequencies and could be related to lactose vehicle inhalation. The most frequent laboratory abnormalities in Phase 3 treatment studies included elevations of liver enzymes and CPK, lymphopenia, and neutropenia. These were reported in similar proportions of zanamivir and lactose vehicle placebo recipients with acute influenza-like illness (Relenza 2003).

However, in children aged 5 to 12 years, nasal signs and symptoms (zanamivir 20 %, placebo 9 %), cough (zanamivir 16 %, placebo 8 %), and throat/tonsil discomfort and pain (zanamivir 11 %, placebo 6 %) were reported more frequently with zanamivir than placebo. In a subset with chronic respiratory disease, lower respiratory adverse events (described as asthma, cough, or viral respiratory infections which could include influenza-like symptoms) were reported in 7 out of 7 zanamivir recipients and 5 out of 12 placebo recipients.

The following adverse reactions have been identified during post-marketing use of zanamivir, but it is not possible to reliably estimate their frequency or establish a cause relationship to zanamivir exposure (Relenza 2003):

- Allergic or allergic-like reaction, including oropharyngeal oedema.
- Arrhythmias, syncope.
- Seizures.
- Bronchospasm, dyspnoea

Zanamivir has not been studied in pregnant women. In animal studies, zanamivir has not been shown to cause birth defects or other problems.

In rats, zanamivir is excreted in milk, but zanamivir has not been studied in nursing mothers and there is no information as to the possible excretion of zanamivir in human milk.

Efficacy

Inhaled zanamivir reduces the median time to alleviation of major influenza symptoms by up to 2.5 days if taken within 48 h of symptom onset. These benefits appear to be particularly marked in severely ill patients and in individuals ≥ 50 years of age, who have underlying illnesses, or who are considered high risk. Patients with a lower temperature or less severe symptoms appear to derive less benefit from treatment with zanamivir.

When used for prophylaxis, zanamivir significantly reduces the number of families with new cases of influenza compared with placebo, and prevented new cases of influenza in long-term care facilities.

Treatment

The first clinical experience with zanamivir included patients from separate randomised, double-blind studies in 38 centres in North America and 32 centres in Europe in 1994-1995. These studies demonstrated approximately a one-day reduction in the time to alleviation of symptoms in treated patients (4 vs. 5 days) (Hayden 1997). An even larger treatment benefit (3 days) was seen in patients who had severe symptoms at entry (Monto 1999). A 3 day treatment benefit was also observed in patients aged > 50 years, compared with 1 day in patients aged < 50 years. In "high-risk" patients there was a treatment benefit of 2.5 days (Monto 1999). In addition, zanamivir has been shown to be effective in patients at risk of developing influenza-related complications such as age ≥ 65 years and the presence of underlying chronic disease including asthma, chronic obstructive pulmonary disease, cardiovascular disease, diabetes mellitus, and immunocompromise (Lalezari 2001).

Influenza infections may lead to respiratory tract complications that result in antibiotic treatment. A meta-analysis of 7 clinical trials reported that 17 % of placebo recipients developed a respiratory event leading to antibiotic use, mainly for acute bronchitis or acute sinusitis, whereas among zanamivir-treated patients the incidence of respiratory events leading to the use of antimicrobials was 11 % (Kaiser 2000b). However, these findings have not remained unquestioned. In the setting of a large managed care plan ($> 2,300$ patients treated), the patterns of influenza complications were found to be similar in zanamivir-treated and untreated patients (Cole 2002).

Prophylaxis

A series of randomised trials have proven the efficacy of zanamivir in the prevention of influenza. In a study involving healthy adults, 10 mg once a day or placebo was administered by oral inhalation at the start of the influenza outbreak. Prophylaxis continued for a 4-week period. Zanamivir was 67 % efficacious in preventing clinical influenza (6 % [34/554] clinical influenza in the placebo group vs. 2 % [11/553] in the zanamivir group) and 84 % efficacious in preventing illnesses with fever (Monto 1999b).

Another clinical trial enrolled families with two to five members and at least one child who was five years of age or older. As soon as an influenza-like illness developed in one family member, the family received either zanamivir (10 mg zanamivir inhaled once daily for 10 days) or placebo. In the zanamivir families, 4 % of families had at least one new influenza case, compared with 19 % in the placebo fami-

lies. The median duration of symptoms was 2.5 days shorter in the zanamivir group than in the placebo group (5.0 vs. 7.5 days) (Hayden 2000). A similar risk reduction was shown in a study where zanamivir was administered after close contact with an index case of influenza-like illness (Kaiser 2000).

In a study of inhaled zanamivir for the prevention of influenza in families, 4 % of zanamivir versus 19 % of placebo households had at least 1 contact who developed symptomatic, laboratory-confirmed influenza (81 % protective efficacy). The protective efficacy was similarly high for individuals (82 %) and against both influenza types A and B (78 % and 85 %, respectively, for households) (Monto 2002).

Children

In a trial on children aged five to twelve years, zanamivir reduced the median time to symptom alleviation by 1.25 days compared with placebo. Zanamivir-treated patients returned to normal activities significantly faster and took significantly fewer relief medications than placebo-treated patients (Hedrick 2000).

Zanamivir is therefore safe in children – if they can take it. Children, especially those under 8 years old, are usually unable to use the delivery system for inhaled zanamivir appropriately (not producing measurable inspiratory flow through the diskhaler or producing peak inspiratory flow rates below the 60 l/min considered optimal for the device). As a lack of measurable flow rate is related to inadequate or frankly undetectable serum concentrations, prescribers should carefully evaluate the ability of young children to use the delivery system when considering prescription of zanamivir. When zanamivir is prescribed for children, it should be used only under adult supervision and with attention to proper use of the delivery system (Relenza 2003).

Special Situations

Special settings in which zanamivir has been used include acute lymphoblastic leukemia (Maeda 2002) and allogeneic stem cell transplantation (Johny 2002). The second report found no toxicity attributable to zanamivir and rapid resolution of influenza symptoms. There was no mortality due to influenza in these patients.

Avian Influenza Strains

In a study performed on mice in 2000, zanamivir was shown to be efficacious in treating avian influenza viruses H9N2, H6N1, and H5N1 transmissible to mammals (Leneva 2001).

Resistance

Development of resistance is rare. To date, no virus resistant to zanamivir has been isolated from immunocompetent individuals after treatment. In addition, all zanamivir-resistant strains selected in vitro to date have diminished viability. Known resistance mutations are both influenza virus subtype and drug specific (McKimm-Breschkin 2003).

There is evidence for different patterns of susceptibility and cross-resistance between neuraminidase inhibitors (Mishin 2005, Yen 2005), but no studies have so far evaluated the risk of emergence of cross-resistance in clinical practice.

Drug Interactions

Zanamivir is administered via inhalation and the low level of absorption of the drug results in low serum concentrations and modest systemic exposure to zanamivir after inhalation. Zanamivir is not metabolised, and the potential for clinically relevant drug-drug interactions is low (Cass 1999b). Zanamivir is not a substrate nor does it affect cytochrome P450 (CYP) isoenzymes (CYP1A1/2, 2A6, 2C9, 2C18, 2D6, 2E1, and 3A4) in human liver microsomes (Relenza 2003). There is no theoretical basis for expecting metabolic interactions between zanamivir and other co-administered compounds (Daniel 1999).

Recommendations for Use

- Zanamivir is indicated for the treatment of uncomplicated acute illness due to influenza A and B viruses in adults and paediatric patients (EU: 12 years or older; US: 7 years and older) who have been symptomatic for no more than 2 days.
- Zanamivir is not recommended for the treatment of patients with underlying airways disease (such as asthma or chronic obstructive pulmonary disease).

Zanamivir (Relenza[®]) is delivered by inhalation because of its low oral bioavailability. Each Relenza[®] Rotadisk contains 4 double-foil blisters and each blister contains 5 mg of zanamivir (plus 20 mg of lactose which contains milk proteins). The contents of each blister are inhaled using a plastic device called a “Diskhaler”. Here, a blister is pierced and zanamivir is dispersed into the air stream when the patient inhales through the mouthpiece. The amount of drug delivered to the respiratory tract depends on patient factors such as inspiratory flow.

Patients should be instructed in the use of the delivery system, and instructions should include a demonstration – which may be difficult in daily medical practice. When prescribed for children, zanamivir should only be used under adult supervision and instruction.

There has been concern over the ability of elderly people to use the inhaling device for zanamivir. A study of 73 patients (aged 71 to 99 years) from wards providing acute elderly care in a large general hospital found that most elderly people could not use the inhaler device and that zanamivir treatment for elderly people with influenza was unlikely to be effective (Diggory 2001).

Dosage

The recommended dose of zanamivir for the treatment of influenza in adults and paediatric patients aged 7 years and older is 10 mg bid (= twice daily 2 consecutive inhalations of one 5-mg blister) for 5 days.

On the first day of treatment, two doses should be taken at least 2 hours apart. On the following days, doses should be taken about 12 hours apart.

No dosage adjustment is required in patients with renal impairment (Cass 1999a).

Patients with pulmonary dysfunction should always have a fast-acting bronchodilator available and discontinue zanamivir if respiratory difficulty develops.

Summary

Trade name: Relenza®

Drug class: Neuraminidase inhibitor.

Manufacturer: GlaxoSmithKline.

Indications: zanamivir is indicated for the treatment of uncomplicated acute illness due to influenza A and B viruses in adults and paediatric patients (EU: 12 years or older; US: 7 years and older) who have been symptomatic for no more than 2 days.

Standard Dosage for Treatment: 10 mg bid (= twice daily 2 consecutive inhalations of one 5-mg blister) for 5 days.

Standard Dosage for Prophylaxis: in most countries, zanamivir has not been approved for prophylaxis.

Pharmacokinetics: 10 to 20 percent of the active compound reaches the lungs, the rest is deposited in the oropharynx. 4 % to 17 % of the inhaled dose is systemically absorbed. Peak serum concentrations are reached within 1 to 2 hours. Limited plasma protein binding (< 10 %). Excretion of the unchanged drug in the urine. Serum half-life after administration by oral inhalation is 2.5 to 5.1 hours.

Warning: zanamivir is not recommended for the treatment of patients with underlying airways disease (such as asthma or chronic obstructive pulmonary disease).

Interactions: no clinically significant pharmacokinetic drug interactions are predicted based on data from in vitro studies.

Side effects: zanamivir has a good safety profile and the overall risk for any respiratory event is low.

Patient information: the use of zanamivir for the treatment of influenza has not been shown to reduce the risk of transmission of influenza to others.

There is a risk of bronchospasm, especially in the setting of underlying airways disease, and patients should stop zanamivir and contact their physician if they experience increased respiratory symptoms during treatment such as worsening wheezing, shortness of breath, or other signs or symptoms of bronchospasm. A patient with asthma or chronic obstructive pulmonary disease must be made aware of the risks and should have a fast-acting bronchodilator available.

Patients scheduled to take inhaled bronchodilators at the same time as zanamivir should be advised to use their bronchodilators before taking zanamivir.

Store at 25° C (77° F); excursions permitted at 15° to 30° C (59° to 86° F).

Internet sources:

USA: <http://influenzareport.com/link.php?id=5>

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Index

- A**
- Adsorption 90
- Aerosols 93
- Age distribution 23
- Alveoli 93
- Amantadine 173, 188
- Drug interactions 190
- Efficacy 190
- Pharmacokinetics 189
- Resistance 188, 190
- Summary 192
- Toxicity 189
- Anseriformes 53
- Anticholinergic-like effects 173
- Antigenic drift 18, 52
- Antigenic shift 18, 25, 52
- Antiviral drugs *See* Drugs
- Aquatic birds 53
- Attachment 51
- Avian influenza *See* Influenza, Avian
- B**
- Binding *See* Adsorption
- Bronchospasm 171
- C**
- Cat 29
- Cell culture 153
- Charadriiformes 53
- Ciliated cells 93
- Cleavage 95
- Clinical course 23
- Clinical presentation 160
- avian 56
- CNS side effects 189
- Complications 161
- cardiac 163
- in HIV-infected patients 164
- Complement fixation 154
- Contact tracing 180
- Containment 35
- Cough 160
- Croup 163
- Cytokines 97
- Cytopathic effects 98
- D**
- Death toll 34
- Diagnosis
- suspected human AI 156
- Differential diagnosis 156
- avian 58
- Discharge policy 180
- Disinfection 122
- Dizziness 173
- Droplets 93
- Drugs 30, 35, 115, 170, 188
- Resistance 172, 173
- E**
- EIA 152, 155
- Embryonated egg culture 153
- Entry
- viral 90, 93
- Epidemic 17
- Epidemiology
- Poultry 66
- Epithelial cells 93
- F**
- Failure of recovery 163
- Fatality rate 23
- Fatigue 160
- Fever 97, 160
- Feverishness 160
- Funding 119
- G**
- Gram staining 162
- Guillain-Barré syndrome 133
- H**
- H5 48
- H5N1 22, 28
- Symptoms 99
- H7 48
- Haemagglutination inhibition 154
- Haemagglutinin 50, 87, 88
- Haemophilus influenzae 87, 162

Headache 160
 Host defense 93
 Host factors 92
 Host response 97
 HPAI
 Control measures 68
 HPAIV 48
 Hygiene 122

I

IgA 154
 IgG 154
 IgM 154
 Immune response
 cellular 103
 humoral 102
 Immunochromatography 155
 Immunofluorescence 152, 155
 Immunology 100
 Influenza
 avian 48
 avian, in humans 164
 Insomnia 173
 Internalisation 51
 Isolation methods 153

L

Laboratory diagnostic 113
 avian 59
 Laboratory findings 150
 Legal issues 118
 Loss of appetite 160
 LPAIV 48

M

M1 protein 51
 M2 inhibitors 173
 M2 protein 51, 89
 Macrophages 93
 Mammals 29
 Matrix protein 87
 MDCK 130
 Mock vaccines 140
 Mortality shift 21
 Mucus 93
 Mutation 25
 Myalgia 160
 Myositis 163

N

Nasal congestion 160
 Natural hosts 53
 Natural reservoir 26
 Nausea 171
 Nervousness 173
 Neuraminidase 50, 87, 89
 Neuraminidase inhibitors 170
 Nomenclature 50
 Non-structural protein 87
 NS1 90
 NS2 90

O

Oseltamivir 172, 194
 Drug interactions 200
 Efficacy 197
 Pharmacokinetics 195
 Resistance 199
 Summary 201
 Toxicity 196

P

Pandemic 17, 34
 1918 21
 1957 22
 1968 22
 Phases 111
 Risk 71
 Simulation 117
 Pathogenesis 54, 92
 Pathology
 avian 57
 Patient management 150
 Pneumonia
 bacterial 162
 mixed 162
 primary 162
 Preparedness
 pandemic 110
 Prophylaxis 30, 31, 180

Q

Qinghai Lake 29

R

Rapid tests 154
 RdRp 52

Reassortment	25
Relenza	<i>See</i> Zanamivir
Replication	96
Replication cycle	90
Reye's Syndrome	164
Rhinitis	160
Ribonucleoprotein	87
Rimantadine	173, 207
Drug interactions	210
Efficacy	209
Pharmacokinetics	208
Resistance	210
Summary	211
Toxicity	208
Risk communication	117, 122
RNP	52
RT-PCR	152, 155
Real-time	157

S

Serology	153
Shedding	91
Skin/hypersensitivity reactions	171
Social distancing	120
Sore throat	160
Specimen collection	150
Sputum culture	162
Staphylococcus aureus	87, 162
Stockpiling	115
Stone marten	29
Streptococcus pneumoniae	87, 162
Surveillance	112, 120, 151

T

Tamiflu	<i>See</i> oseltamivir
Tests	
Rapid	154
Toxic shock syndrome	163
Tracing	121
Transmission	27, 100
between birds	62
Prophylaxis	179
to humans	63
to other Mammals	65
Treatment	31, 33, 170
Children	177
human H5N1 influenza	178
impaired liver function	177

impaired renal function	177
Pregnancy	178
seasonal human influenza	174
Seizure disorders	178

U

Uncoating	90
-----------------	----

V

Vaccination	30, 33
Guidelines	134
Poultry	69
Seasonal	117
Vaccines	36, 114, 127
Adjuvants	131
Antigen sparing	138
Cell culture	130
Companies	137
Contraindications	135
Development	127
DNA	131
Dosage	136
Efficacy	132
M2	131
pandemic	139
Production	127
Reverse genetics	131
Side effects	133
Types	129
Viral factors	93
Virology	50, 87
Vomiting	171

W

Weakness	160
----------------	-----

Z

Zanamivir	172, 213
Drug interactions	218
Efficacy	216
Pharmacokinetics	214
Resistance	217
Summary	219
Toxicity	215