EMERGING INFECTIOUS DISEASES

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Avian Influenza

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Winslow Homer (1836-1910). Right and Left (1909).
Oil on canvas (0.718 m × 1.229 m).
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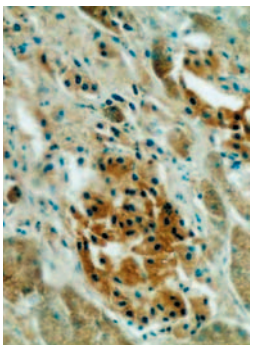

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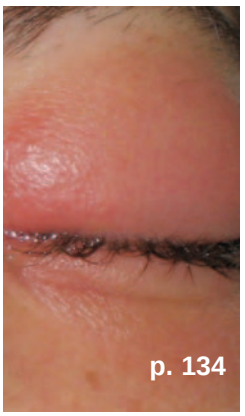
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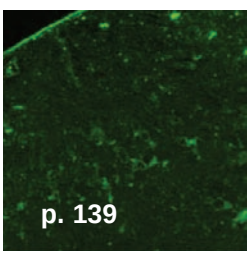
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Influenza Revisited

Jeffery K. Taubenberger* and David M. Morens†

This issue of Emerging Infectious Diseases includes a group of invited articles addressing pandemic influenza. Over the past 2 years, concerns about a new influenza pandemic caused by either an epizootic avian strain, such as H5N1, or by some other influenza virus have engaged top virologists, epidemiologists, and policymakers as well as the press and public (*1*,*2*). However, many scientific questions about the risk of a pandemic remain unanswered, and as science attempts to catch up on decades of relative neglect, fear and speculation have begun to mount. Such speculation has led to what the press has called "hysteria" in private stockpiling of antiviral drugs; this panic has even been compared to the widespread fear of an atomic bomb attack that gripped the United States in the late 1950s and early 1960s, when many citizens built and stocked underground fallout shelters.

In this climate, scientific and public health communities must continually update and review what is known about the risk of pandemic influenza and about options to prevent and control it. This group of articles is intended to serve as a modest database of current knowledge and informed opinion in several key areas, including the history of pandemic influenza and public health responses to it; influenza pathogenesis, natural history, and host immune responses to infection; and influenza prevention and treatment with drugs and vaccines.

Missing from the list of authors in this issue is a man whose insight, effort, and support probably did more to advance our understanding of influenza than the efforts of any other single individual over the past 30 years, John R. LaMontagne, whose untimely death in 2004 was a great loss to the scientific community (for additional information, see http://www3.niaid.nih.gov/about/overview/previous directors/LaMontagne/).

John would have agreed with another visionary scientist, Hermann Pidoux (1808–1882), who observed that "epidemics are the lives of diseases." In an attempt to understand a disease as explosive and fatal as pandemic influenza, the classic emerging/reemerging infectious disease, its history has been self-consciously chronicled for several centuries. The importance of that effort was recognized during the pandemic of 1889 and strongly reinforced by the next pandemic in 1918–1919 (the so-called "Spanish flu," the deadliest pandemic in human history). We review the life cycle of pandemic influenza during the past century, including the pandemics of 1918, 1957, 1968, and 1977, as well as a feared nonpandemic in 1976, looking at pandemics from different angles, questioning whether they are predictable and, if they are, what telltale signs we should be looking for.

The answers to these questions may not be reassuring. The origin of the earliest human influenza virus yet identified, the 1918 pandemic virus, is still a mystery even after genetic sequencing and comparison with other historical and circulating influenza viruses (*3*,*4*). Though clearly descended from an avian virus, the 1918 strain is genetically unlike any other influenza virus examined over the

*Armed Forces Institute of Pathology, Rockville, Maryland, USA; and †National Institutes of Health, Bethesda, Maryland, USA

Guest Editors

Jeffery K. Taubenberger

Dr Taubenberger is chair of the Department of Molecular Pathology at the Armed Forces Institute of Pathology in Rockville, Maryland. His clinical interest is in diagnostic molecular genetic pathology. His research interests include the molecular pathophysiology and evolution of influenza viruses.

David M. Morens

Dr Morens is an epidemiologist with a long-standing interest in emerging infectious diseases, virology, tropical medicine, and medical history. He spent more than 6 years at the US Centers for Disease Control, followed by 17 years at the University of Hawaii. Since 1999, he has worked at the National Institute of Allergy and Infectious Diseases. He is an associate editor of Emerging Infectious Diseases.

past 88 years, which indicates that its immediate origin before the pandemic is an unknown source. Complicating problems of origin, all of the pandemic and epidemic influenza A viruses that have appeared since 1918 have been descendants of it, arising by either genetic drift, reassortment with prevalent avian viruses, or in 1 case (1977) by apparent release from a freezer. Thus, little scientific basis exists for predicting whether the current enzootic/epizootic avian H5N1 virus will become pandemic: none of the known pandemic influenza events of the past 87 years seem to have much in common with the current H5N1 situation.

Another problem is learning about the mechanisms by which influenza A viruses, all of which are believed to be endemic in wild waterfowl, their natural hosts, acquire the capacities to switch hosts, produce diseases in these new hosts, and in some cases, establish the ability to propagate directly between them. While preliminary information about the first 2 of these capacities is gradually becoming known (*5–7*), little has been learned about the third. Thus, predicting whether current H5N1 viruses are moving in the direction of solving the ultimate challenge of host-switching/propagation in humans, or whether they are fundamentally incapable of doing so, is difficult.

Although science may yet offer little in the way of pandemic prediction, understanding the size of the influenza problem and the mechanisms by which influenza viruses cause severe and fatal disease, i.e., pathogenesis, is still important. Such knowledge is fundamental if we expect to prevent and control epidemics using public health measures and clinical therapies. Again, answers are elusive. Although influenza is a leading cause of death worldwide, measuring the total effect of deaths from influenza is impossible, in part because diagnostic records for a key risk group, the elderly, are incomplete and imprecise (*8*). Influenza also kills by different mechanisms such as primary viral pneumonia, secondary bacterial pneumonia in virus-damaged lungs, and an acute respiratory distresslike syndrome possibly associated with overly brisk immune responses, as well as by cardiac and other pathways, and by exacerbating serious chronic diseases such as diabetes mellitus, renal diseases, and congestive heart failure. The problems of understanding influenza occurrence and pathogenesis are therefore complicated by the many different pathways that lead to severe disease and death and by the difficulty in determining the frequency with which these events occur.

Because of these uncertainties and knowledge gaps, establishing effective programs for public health control and personal protection is particularly important. Vaccines and drugs against circulating influenza viruses have been used for decades, but their efficacy in any future pandemic is difficult to predict because, with current knowledge, the causative agent of a future pandemic cannot be known in advance and may well be a novel virus whose susceptibility to existing drugs and vaccines has not been established. Important new technologies allow construction and pretesting of vaccines against all of the known influenza surface glycoproteins (16 hemagglutinins and 9 neuraminidases), although the likelihood that a new pandemic strain would be preventable by such vaccines cannot be known without an ability to predict its antigenic nature. Among additional strategies to overcome this limitation is development of "universal" vaccines based on antigens shared by many, and ideally all, influenza viruses.

The recent H5N1 epizootics in Southeast Asia serve as an important reminder of how few of the key determinants of pandemic influenza are really understood. If there is a single lesson to be learned from the articles in this issue, it is that, as expressed by contributor Anthony Fauci, more research is needed in many areas. We do not know whether pandemic influenza will outpace the increasingly vigorous research to contain it. But the race is on, the stakes are high, and the world is nervously watching.

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Address for correspondence: Jeffery K. Taubenberger, Department of Molecular Pathology, Armed Forces Institute of Pathology, 1413 Research Blvd, Bldg 101, Rm 1057, Rockville, MD 20850-3125, USA; fax. 301-295-9507; email: taubenberger@afip.osd.mil

H5N1 Outbreaks and Enzootic Influenza

Robert G. Webster,*† Malik Peiris,†‡ Honglin Chen,†‡ and Yi Guan†‡

Ongoing outbreaks of H5N1 avian influenza in migratory waterfowl, domestic poultry, and humans in Asia during the summer of 2005 present a continuing, protean pandemic threat. We review the zoonotic source of highly pathogenic H5N1 viruses and their genesis from their natural reservoirs. The acquisition of novel traits, including lethality to waterfowl, ferrets, felids, and humans, indicates an expanding host range. The natural selection of nonpathogenic viruses from heterogeneous subpopulations cocirculating in ducks contributes to the spread of H5N1 in Asia. Transmission of highly pathogenic H5N1 from domestic poultry back to migratory waterfowl in western China has increased the geographic spread. The spread of H5N1 and its likely reintroduction to domestic poultry increase the need for good agricultural vaccines. In fact, the root cause of the continuing H5N1 pandemic threat may be the way the pathogenicity of H5N1 viruses is masked by cocirculating influenza viruses or bad agricultural vaccines.

Influenza is an ancient disease that has infected humans at irregular intervals throughout recorded history (*1*). While the 1918 "Spanish" influenza is the best recorded catastrophic influenza pandemic, similarly severe pandemics occurred earlier, when the human population of the world was much smaller, and they will occur again. Our challenge is to understand all aspects of the influenza virus, the hosts and their response, and the virus' global impact so that we may be better prepared to face the inevitable next influenza pandemic.

The influenza virus that appears most threatening is the avian H5N1 strain that since 2003 has infected >130 persons in Vietnam, Thailand, and Cambodia and has killed more than half of them. Nonetheless, the H5N1 influenza threat is viewed with disturbing complacency; a frequently heard statement is "since the virus has not adapted to continuing human-to-human transmission by now, it is unlikely to do so in the future." Such complacency is akin to living on a geologic fault line and failing to take precautions against earthquakes and tsunamis.

*St. Jude Children's Research Hospital, Memphis, Tennessee, USA; †University of Hong Kong, Hong Kong, SAR, China; and ‡Shantou University Medical College, Shantou, Guangdong, China

The Source

Influenza A viruses are perpetuated in the wild birds of the world, predominantly in waterfowl, in which the 16 subtypes (which differ by 30% in their hemagglutinin [HA] nucleotide homology) coexist in perfect harmony with their hosts (*2*,*3*) (Figure 1). In these natural hosts, the viruses remain in evolutionary stasis, showing minimal evolution at the amino acid level over extended periods. This fact indicates that the influenza-bird association is ancient; this lack of change is surprising because influenza viruses are segmented, negative-stranded RNA viruses that have no quality-control mechanisms during replication and are highly prone to variation. After transfer to a new type of host, either avian or mammalian, influenza viruses undergo rapid evolution. However, all 16 HA subtypes, including H5 and H7, have until recently been considered to be benign in their natural hosts. This benign equilibrium between the influenza virus and its host may have changed.

Genesis of the H5N1 Virus

Before 1997, no evidence had indicated that H5 influenza viruses could infect humans and cause fatal disease. The H7 influenza viruses were known to cause conjunctivitis in humans, and serologic studies provided evidence of subclinical human infection with the subtypes prevalent in avian live poultry markets (*4*). The precursor of the H5N1 influenza virus that spread to humans in 1997 was first detected in Guangdong, China, in 1996, when it caused a moderate number of deaths in geese and attracted very little attention (*5*). This goose virus acquired internal gene segments from influenza viruses later found in quail (A/Quail/HK/G1/97 [H9N2]) and also acquired the

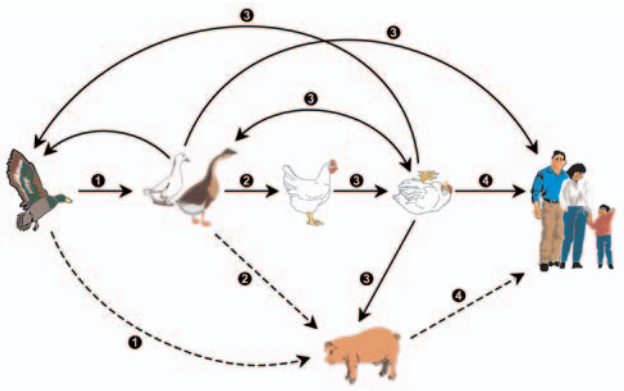

Figure 1. Emergence of H5N1 influenza virus and control options. A nonpathogenic H5 influenza virus is believed to have spread to domestic ducks and geese, then to domestic chickens. In chickens, the H5 virus became highly pathogenic before it was transferred back to domestic ducks and geese. The highly pathogenic H5 virus reassorted its genome with those of other influenza viruses in aquatic birds, and the resulting viruses spread to domestic poultry farms, humans, and occasionally to pigs. These viruses acquired mutations in their PB2, HA, NA, and NS genes that made them lethal to domestic and wild waterfowl and humans. Solid lines, transmission demonstrated; dotted lines, transmission postulated but not demonstrated. Multiple opportunities exist for control of 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 the use of vaccines that prevent disease and transmission; 4) prevent contact between poultry, pigs, and humans and make vaccines and antiviral drugs available.

neuraminidase gene segment from a duck virus (A/Teal/HK/W312/97 [H6N1]) before the goose virus became widespread in live poultry markets in Hong Kong and killed 6 of 18 infected persons (*6,7*). This H5N1 virus was eradicated by culling all domestic poultry in Hong Kong, and the genotype has not been detected since that time. However, different reassortants continued to emerge from goose and duck reservoirs (*8*) that contained the same H5 HA glycoprotein but had various internal genes. The H5N1 viruses continued to evolve, and in late 2002, a single genotype was responsible for killing most wild, domestic, and exotic waterfowl in Hong Kong nature parks (*9,10*). This genotype of H5N1 spread to humans in Hong Kong in February 2002, killing 1 of 2 infected persons (*11*), and was the precursor of the Z genotype that became dominant. The Z genotype spread in an unprecedented fashion across Southeast Asia, affecting Vietnam, Thailand, Indonesia, Cambodia, Laos, Korea, Japan, China, and later Malaysia. Further analysis showed that the H5N1 influenza viruses that caused outbreaks in poultry in Japan and Korea were genetically different from those in the other countries (the V genotype) (*12,13*). The phylogeny of the recent Z genotype viruses showed that viruses isolated in Vietnam and Thailand formed a cluster that remained distinct from those isolated in Indonesia.

To date, >140 million domesticated birds have been killed by the virus or culled to stem its spread; as of December 2005, >130 persons have been infected in Vietnam, Thailand, Indonesia, Cambodia, and China, and 70 have died (42 in Vietnam, 14 in Thailand, 8 in Indonesia, 4 in Cambodia, and 2 in China). These recent H5N1 influenza viruses have acquired the unprecedented and disturbing capability to infect humans; to cause neurotropic disease and a high proportion of deaths in waterfowl in nature; to cause death in and be transmitted among felid species, including domestic cats (*14*); and to cause neurotropic disease and death in ferrets and mice (*15*). These incremental changes intensify concern about this H5N1 virus' pandemic potential. These traits are likely to have been acquired initially by reassortment in 2001 and 2002, when a plethora of different genotypes were detected in poultry markets and later in farms in Hong Kong (*13*). These genes were presumably acquired from viruses found in waterfowl in Southeast Asia, but the actual gene donors have not yet been identified. Since late 2002, the Z genotype has become dominant, but phylogenetically distinguishable viruses have continued to cocirculate in Indonesia and western China. These characteristics have been acquired mainly through mutations in the RNA polymerase (PB2) gene, insertions in the HA gene, and deletions in the NA and nonstructural (NS) genes. Thus, the H5N1 viruses continue to evolve, initially by reassortment and more recently by mutation and deletion (*16,17*). While most H5N1 influenza viruses isolated from avian species in Asia since 1997 are highly pathogenic in gallinaceous poultry, they show heterogeneous pathogenicity in other species.

In domestic ducks, the pathogenicity of the H5N1 viruses varies from high to nonpathogenic. In ferrets, most avian isolates replicate and cause respiratory tract infection, while a few strains are highly pathogenic and neurotropic (causing hind leg paralysis), and the virus has been isolated from the brain (*15*). In contrast, all isolates from humans are highly pathogenic to ferrets. A similar pattern is found in experimental infection of mice, in which most avian isolates cause respiratory infection.

Mechanisms of Spread

Were the highly pathogenic H5N1 viruses transferred within and between countries by persons, poultry, or fomites? In previous outbreaks of highly pathogenic H5 and H7 infection in multiple countries, the spread was directly attributable to humans. The main way influenza

virus is spread in poultry is by movement of poultry and poultry products; establishing good biosecurity measures on poultry farms is therefore an important defense. The poultry industry is a huge, integrated complex in Asia, and a number of firms have branches in China, Vietnam, Thailand, and Indonesia. Nonetheless, the involvement of multiple lineages of H5N1 argues against human-mediated spread from a single source. Live poultry markets are an amplifier and reservoir of infection (*18*) and probably play a role in the maintenance and spread of the virus in the region. However, a number of other factors unique to affected Asian countries make control difficult. Backyard flocks are common in the region, and these domesticated birds are not subject to any biosecurity measures. Fighting cocks are prized possessions and are often transported long distances. Fighting cocks may also play a role in the spread of infection and in transmission to humans. Many of the affected countries have a weak veterinary infrastructure and are facing highly pathogenic avian influenza outbreaks for the first time. The migrant ducks that commonly wander through rice fields scavenging fallen rice seeds are another potent mechanism for the spread of infection.

Role of Domestic Ducks

After late 2002, when H5N1 viruses had killed waterfowl in Kowloon Park in Hong Kong, most avian H5N1 isolates isolated in Vietnam, Thailand, and Indonesia were highly pathogenic to chickens and domestic ducks. However, by late 2003 and early 2004, some avian isolates were nonpathogenic to ducks but retained their pathogenicity to chickens (*19*). Genetic analysis of these isolates showed evidence of multiple variants within single specimens (*20*). On Madin-Darby canine kidney (MDCK) cells, these viruses formed a mixture of small and large plaques that had different biologic properties. Viruses that formed large plaques were usually highly pathogenic to ducks and ferrets, whereas viruses that formed small plaques were usually nonpathogenic to both birds and ferrets. Some virus isolates formed small plaques that were pathogenic to ducks. Thus, plaque size was not a marker of pathogenicity. When ducks were orally infected with the original mixed population of H5N1 viruses, most birds died, but some excreted virus for an extended period (up to 17 days); during this time, viruses that were nonpathogenic to ducks were selected. Serologic testing of these ducks showed hemagglutination inhibition (HI) and neutralizing antibodies against the original dominant virus in the mixture; thus, immune clearance had caused the selection of the minor variants. The viruses shed on day 17 had become nonpathogenic to ducks, although they remained highly pathogenic to chickens. Sequence analysis of the HA showed that these viruses differed from the original dominant virus at multiple amino acids and were antigenically distinguishable in HI tests. Therefore, H5N1 viruses circulating in avian populations in Southeast Asia are clearly heterogeneous. Notably, this phenomenon has repeatedly been reported for other influenza viruses that are in the process of altering their interspecies transmission, including European avian H1N1 viruses that were transmitted to pigs (*21*), H9N2 viruses that were transmitted to pigs and humans, and now H5N1 viruses that are transmitted from ducks to humans. How these mixtures of codominant viruses are generated in a quasispecies is unresolved. Suggested mechanisms include mutator mutations or partial heterozygotes, but a satisfactory explanation is not available (*22*).

A subdominant population of H5N1 viruses is presumably selected in ducks after the immune response clears the dominant virus. The subdominant population appears to be uniformly nonpathogenic to ducks, as if this is the natural situation for influenza in the duck. Whether further selection will occur against the polybasic cleavage site in the HA and the pathogenicity determining sites in PB2 and NS remains to be seen. These viruses' loss of pathogenicity to ducks, but retention of pathogenicity to chickens and presumably to humans, has been a problem associated with their eradication. In Vietnam, for example, disease signs were used as the criteria for identifying H5N1 infection in ducks. Thus, the duck has become the Trojan horse of highly pathogenic H5N1 influenza in Asia (*20*).

Role of Migratory Birds

Migratory waterfowl are generally believed to be the main reservoir of all 16 subtypes of influenza A viruses, including H5 and H7 subtypes. However, less agreement is found regarding the role of migratory waterfowl in the initial spread of highly pathogenic H5N1 viruses across eastern Asia in 2003. The isolation of highly pathogenic H5N1 from herons, egrets, and peregrine falcons in Hong Kong in 2003 and 2004 leaves no doubt that wild migratory birds can be infected and may spread disease to local poultry flocks. The outbreak in Qinghai Lake (*16,17*) proves that these highly pathogenic H5N1 influenza viruses are transmissible among migratory waterfowl. The migration route of shorebirds in the east Asian-Australasian flyway does overlap the areas that have had H5N1 outbreaks, although the virus has been notably absent in Taiwan, Malaysia (except for occasional outbreaks near the Thai border), and western Australia (Figure 2). The role of migratory birds in the transmission and spread of highly pathogenic H5N1 viruses is still unclear. However, the recent outbreak of H5N1 infection in bar-headed geese and other species in Qinghai Lake is a cause for concern because these birds migrate southward to the Indian subcontinent, an area that has apparently not been affected by H5N1 avian influenza. If the virus were to become entrenched in India, its

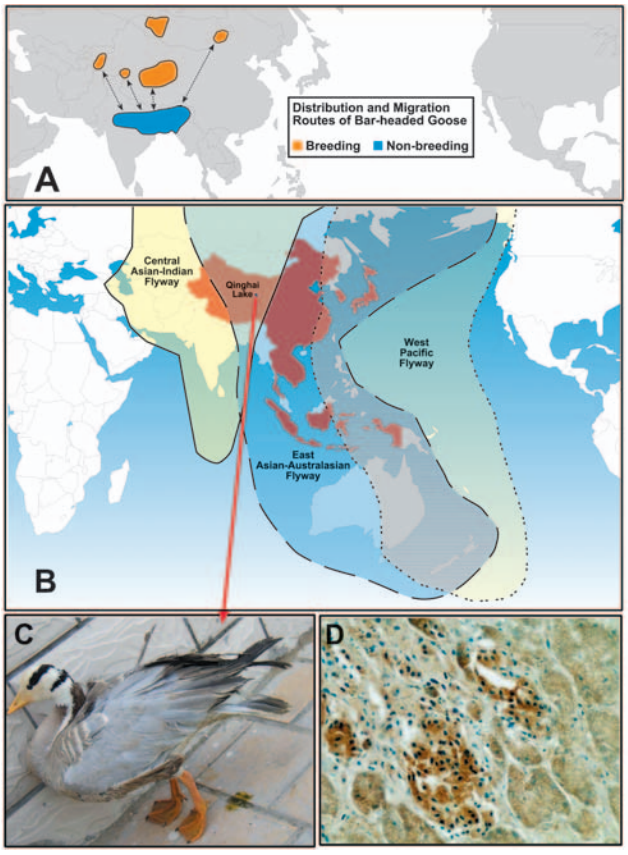

Figure 2. Migration routes of Asian birds. A) Distribution and migration routes of bar-headed geese (courtesy of P. Leader). B) The Asia-Pacific region contains >240 species of migratory birds. The 3 flyways run primarily in a north-south direction, overlapping and extending from Australia/New Zealand to India, Central Asia, and Siberia. The outbreak of highly pathogenic (HP) H5N1 in migratory waterfowl at Qinghai Lake, China, affected primarily bar-headed geese (*Anser indicus*); however, other species, including gulls and ducks, were affected (*16*,*17*). The outbreak started in early May 2005, and by June >5,000 birds had died. The birds exhibited neurologic signs, inability to stand, diarrhea, and death. Systemic infection was detected in all organs tested. C) Bar-headed goose infected with HP H5N1 influenza virus. D) Immunostain of goose pancreas, using H5 monoclonal antibodies (magnification ×400). (C and D, courtesy of H. Chen). Countries shown in red have had outbreaks of HP H5N1 since 2004. The geographic range of H5N1 may be extended by bar-headed geese or by ducks that are less susceptible to lethal infection.

geographic range would be substantially extended, and the pandemic threat would increase accordingly (*17*). A mutation in the PB2 gene (residue E627K) associated with pathogenicity in mammals (*16,17*) has been found in viruses isolated from birds in Qinghai Lake; this finding has caused concern that this mutation will be transferred to other migratory birds (e.g., wild ducks) and will be spread because not all infected birds die.

Although culling domestic poultry to contain the spread of highly pathogenic H5N1 virus is considered an acceptable agricultural practice, culling migratory birds is not acceptable to any international authority (Food and Agriculture Organization of the United Nations [FAO], the World Organization for Animal Health [OIE], the World Health Organization [WHO]). The idea of culling migratory birds must be strongly discouraged, for it could have unknown ecologic consequences. Instead, since highly pathogenic H5N1 has been demonstrated in migratory birds, the poultry industries of the world must adapt measures such as increased biosecurity (Figure 1), the use of vaccines, or both.

Early detection and aggressive control measures allowed Japan, South Korea, and Malaysia to eradicate H5N1 virus soon after its introduction into those countries' poultry flocks, demonstrating that rapid and determined responses can keep the virus from gaining a foothold. In other countries in Asia, delayed detection and response caused the virus to become entrenched across a wide region, and eradication at this stage has become a formidable undertaking.

Agricultural Vaccines

The need for H5N1 vaccines for domestic poultry is increasing. Adopting a policy to use vaccines in poultry is an important decision for agricultural authorities in countries such as Thailand (a major poultry exporter) and Vietnam. Both countries are investigating their specific needs. While considerable data exist on the efficacy of influenza vaccines in domestic chickens, little comparable information is available regarding ducks. The pros and cons of the use of vaccines in poultry have been reviewed (*23*). Current technologies permit discrimination between vaccinated and naturally infected birds; however, vaccines are not standardized on the basis of antigen content. "Good" and "bad" agricultural vaccines are in use.

Good Agricultural Vaccines

Good agricultural vaccines provide protection from disease despite lack of a close antigenic match between the vaccine and circulating strain and reduce the virus load below the level of transmissibility. They do not provide sterilizing immunity: vaccinated birds may excrete low levels of virus after challenge infection. Sentinel unvaccinated birds are kept in each house to monitor for virus shedding, antigenic drift, or both.

Bad Agricultural Vaccines

Bad agricultural vaccines prevent disease signs but do not prevent shedding of transmissible levels of virus. They also promote undetected spread of virus on farms and to live poultry markets and promote antigenic drift. China

and Indonesia have adopted poultry vaccination to control H5N1, and Vietnam has begun vaccine trials in poultry. However, the resurgence of H5N1 in Indonesian poultry and pigs (*24*) and the detection of H5N1 in apparently healthy birds in live poultry markets in China (*17*) suggest that some vaccines are of suboptimal quality or that coinfection masks disease. The adoption of a vaccine strategy for H5N2 virus in Mexico in the 1980s reduced disease signs but has not eliminated the H5N2 virus from the region; instead, vaccination may have contributed to the virus' widespread presence in Central America and to its antigenic drift (*25*).

H9N2 and Cross-protection

The clinical signs of infection with highly pathogenic H5N1 virus may be masked by cross-protection by other influenza subtypes, but this fact is often overlooked. During the initial outbreak of highly pathogenic H5N1 in Hong Kong in 1997, chickens in the live poultry markets exhibited no disease signs, yet samples from apparently healthy chickens, ducks, and quail showed highly pathogenic H5N1 in each of the poultry markets surveyed (*26*). Surveillance showed that multiple influenza subtypes were cocirculating, including 2 lineages of H9N2, the first represented by the G1 lineage (A/Quail/Hong Kong/G1/97 [H9N2]) and the other by G9 (A/Chicken/Hong Kong/G9/97 [H9N2]). The G1 lineage has the same 6 internal gene segments as the index H5N1 human isolate (A/Hong Kong/156/97 [H5N1]) and is believed to have been the donor of these genes during reassortment that produced the original H5N1 human strain in 1997 (*27*). In laboratory studies, chickens previously infected with H9N2 (A/Quail/Hong Kong/G1/97 [H9N2]) were protected from disease signs and death when challenged with highly pathogenic H5N1, but the chickens shed H5N1 virus in their feces (*28*). Further studies in inbred chickens established that the cross-protection was due to cell-mediated immunity and that it could be transferred by CD8+ T cells but not by antibodies (*29*).

The possible effect of cocirculating influenza viruses on the pathogenicity of highly pathogenic H5N1 in Vietnam, Thailand, and elsewhere in Asia has not been resolved. To date, no other subtypes of influenza A viruses have been reported in poultry in Vietnam or Thailand. Surveillance of live poultry in Hong Kong and in Nanchang (*30*) suggests that other influenza A viruses are cocirculating in live poultry markets and on duck farms. Definitive information is required to understand the ecology of influenza and the possible masking of disease signs caused by H5N1.

Conclusion

Conventional wisdom about pandemic influenza holds that a pandemic is inevitable and that the only question remaining is "When?" The H5N1 virus continues to evolve and spread, with additional human infections occurring in Vietnam, Cambodia, Indonesia, China, and Thailand. If this virus acquires human-to-human transmissibility with its present fatality rate of 50%, the resulting pandemic would be akin to a global tsunami. If it killed those infected at even a fraction of this rate, the results would be catastrophic. While the high pathogenicity of the Qinghai bar-headed goose isolate is a continuing threat to poultry and humans, perhaps the most insidious threat comes from unobserved transmission through wild and domestic ducks. The isolation of H5N1 virus from bar-headed geese in Qinghai Lake in southern China in 2005 originated from unobserved infection in poultry markets and suggests that highly pathogenic H5N1 viruses continue to circulate unseen among poultry in China (*17*). We cannot afford simply to hope that human-to-human spread of H5N1 will not happen and that, if it does, the pathogenicity of the virus will attenuate. Notably, the precursor of the severe acute respiratory syndrome (SARS)–associated coronavirus (*31*) repeatedly crossed species barriers, probably for many years, before it finally acquired the capacity for human-to-human transmission, and its pathogenicity to humans was not attenuated. We cannot wait and allow nature to take its course. SARS was interrupted by early case detection and isolation, but influenza is transmissible early in the course of the disease and cannot be controlled by similar means. Just 1 year before the catastrophic tsunami of December 2004, Asian leaders rejected a proposed tsunami warning system for the Indian Ocean because it was too expensive and the risk was too remote. This mistake must not be repeated in relation to an H5N1 avian influenza pandemic. We must use this window of opportunity to prepare and to begin prepandemic implementation of prevention and control measures.[1]

Acknowledgments

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Dr Webster is a professor in the Division of Virology, Department of Infectious Diseases at St. Jude Children's Research Hospital. The major focus of his research concerns

[1]Since this article was written, the H5N1 influenza virus has continued to spread in migratory birds to Turkey, Croatia, and Romania. The global spread of this H5N1 in migratory birds and domestic poultry is inevitable. The question is, "When will it acquire sustained human-to-human transmission?"

influenza viruses in wild aquatic birds and their role in the evolution of new pandemic strains for humans and animals.

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Address for correspondence: Robert G. Webster, St. Jude Children's Research Hospital, Department of Infectious Diseases, Division of Virology, 332 N. Lauderdale, Memphis, TN 38105-2794, USA; fax: 901-523-2622; email: robert.webster@stjude.org

Influenza Pandemics of the 20th Century

Edwin D. Kilbourne*

Three worldwide (pandemic) outbreaks of influenza occurred in the 20th century: in 1918, 1957, and 1968. The latter 2 were in the era of modern virology and most thoroughly characterized. All 3 have been informally identified by their presumed sites of origin as Spanish, Asian, and Hong Kong influenza, respectively. They are now known to represent 3 different antigenic subtypes of influenza A virus: H1N1, H2N2, and H3N2, respectively. Not classified as true pandemics are 3 notable epidemics: a pseudopandemic in 1947 with low death rates, an epidemic in 1977 that was a pandemic in children, and an abortive epidemic of swine influenza in 1976 that was feared to have pandemic potential. Major influenza epidemics show no predictable periodicity or pattern, and all differ from one another. Evidence suggests that true pandemics with changes in hemagglutinin subtypes arise from genetic reassortment with animal influenza A viruses.

Three worldwide (pandemic) influenza outbreaks occurred in the last century. Each differed from the others with respect to etiologic agents, epidemiology, and disease severity. They did not occur at regular intervals. In the case of the 2 that occurred within the era of modern virology (1957 and 1968), the hemagglutinin (HA) antigen of the causative viruses showed major changes from the corresponding antigens of immediately antecedent strains. The immediate antecedent to the virus of 1918 remains unknown, but that epidemic likely also reflected a major change in the antigens of the virus (*1*)

Brief Look Back at the 1918 Pandemic

This notorious epidemic is thoroughly and cogently discussed elsewhere in this issue of Emerging Infectious Diseases (*1*). I wish only to add a few points that are not often emphasized, or even mentioned.

The origin of this pandemic has always been disputed and may never be resolved. However, the observations of trained observers at that time are worth noting because they may bear on later genomic analysis of the recently resurrected 1918 virus nucleotide fragments (*1*) and the abortive "swine flu" epidemic of 1976. In Richard Shope's Harvey lecture of 1936 (*2*), he reviews evidence that in the late summer or early autumn of 1918, a disease not previously recognized in swine, and closely resembling influenza in humans, appeared in the American Middle West. Epidemiologic-epizootiologic evidence strongly suggested that the causative virus was moving from humans to swine rather than in the reverse direction. Similar observations were made on the other side of the world and reported in a little-known paper in the National Medical Journal of China (*3*). In the spring of 1918, influenza in humans spread rapidly all over the world and was prevalent from Canton, China, to the most northern parts of Manchuria and from Shanghai to Szechuan. In October 1918, a disease diagnosed as influenza appeared in Russian and Chinese pigs in the area surrounding Harbin. Thus, epidemiologic evidence, fragmentary as it is, appears to favor the spread of virus from humans to swine, in which it remained relatively unchanged until it was recovered more than a decade later by Shope in the first isolation of influenza virus from a mammalian species.

The virus of 1918 was undoubtedly uniquely virulent, although most patients experienced symptoms of typical influenza with a 3- to 5-day fever followed by complete recovery. Nevertheless, although diagnostic virology was not yet available, bacteriology was flourishing and many careful postmortem examinations of patients by academic bacteriologists and pathologists disclosed bacterial pathogens in the lungs (*4*) However, this was a time when bacterial superinfection in other virus diseases could lead to death; for example, measles in military recruits was often fatal (*4*). This information is important in considering the question of "will there ever be another 1918." To the degree that secondary bacterial infection may contribute to influenza death rates, it should at least be

*New York Medical College, Valhalla, New York, USA

partially controllable by antimicrobial agents, as indeed was the case in 1957.

1957: Asian Influenza (H2N2)

After the influenza pandemic of 1918, influenza went back to its usual pattern of regional epidemics of lesser virulence in the 1930s, 1940s, and early 1950s. With the first isolation of a virus from humans in 1933 (*5*), speculation began about the possible role of a similar virus in 1918. However, believing that this could have been the case was difficult until the pandemic of 1957. This was the first time the rapid global spread of a modern influenza virus was available for laboratory investigation. With the exception of persons >70 years of age, the public was confronted by a virus with which it had had no experience, and it was shown that the virus alone, without bacterial coinvaders, was lethal (*6*).

First Recognition of the Pandemic

In 1957, worldwide surveillance for influenza was less extensive than it is today. However, attentive investigators in Melbourne, London, and Washington, DC soon had the virus in their laboratories (*7*) after the initial recognition of a severe epidemic, followed by the publication in The New York Times of an article in 1957 describing an epidemic in Hong Kong that involved 250,000 people in a short period (*8*). Three weeks later, a virus was recovered from the outbreak and sent to Walter Reed Army Institute for Research in Washington, DC for study.

Nature of the Virus

The virus was quickly recognized as an influenza A virus by complement fixation tests. However, tests defining the HA antigen of the virus showed it to be unlike any previously found in humans. This was also true for the neuraminidase (NA) antigen. The definitive subtype of the Asian virus was later established as H2N2. The new virus had high sialidase/neuraminidase activity, and this activity was more stable than that of earlier strains. Different strains of the Asian virus also differed markedly with respect to sensitivity to either antibody neutralization or nonspecific inhibitors of hemagglutination (*9*). In animal studies, the new H2N2 viruses did not differ in their virulence characteristics from earlier influenza A subtypes. Viral isolates from the lungs of patients with fatal cases showed no discernible differences from those from throat washing isolates of patients without pulmonary involvement within a small circumscribed hospital outbreak (*10*).

Primary Influenza Virus Pneumonia

Although secondary or concomitant bacterial infections of the lung were found to be a prominent feature of fatal cases in 1918 when a specific etiologic agent was sought (*4*), many cases of rapid death and lung consolidation or pulmonary edema occurred in which bacterial infection could not be demonstrated. As influenza persisted as an endemic disease with regional recurrences after the pandemic, lives continued to be occasionally claimed by abacterial pneumonia.

With the arrival of Asian influenza in 1957, the sheer number of cases associated with pandemicity again brought the phenomenon of primary influenza virus pneumonia to the attention of physicians in teaching hospitals. In contrast to the observations in 1918, underlying chronic disease of the heart or lungs was found in most of these patients, although deaths of previously healthy persons were not uncommon. In the case of carefully studied patients at the New York Hospital, rheumatic heart disease was the most common antecedent factor, and women in the third trimester of pregnancy were among those vulnerable (*11*).

Response to Vaccination in an Unprimed Population

The pandemic of 1957 provided the first opportunity to observe vaccination response in that large part of the population that had not previously been primed by novel HA and NA antigens not cross-reactive with earlier influenza A virus antigens. As summarized by Meiklejohn (*12*) at an international conference on Asian influenza held 3 years after the 1957 onslaught of H2N2, more vaccine was required to initiate a primary antibody response than with the earlier H1 vaccines (almost always observed in heterovariant primed subjects). In 1958, 1959, and 1960 (as recurrent infections occurred), mean initial antibody levels in the population increased (i.e., subjects were primed) and response to vaccination was more readily demonstrated. Divided doses given at intervals of $\leq$4 weeks were more beneficial than a single injection. Less benefit was derived from this strategy as years passed. Intradermal administration of vaccine provided no special advantage over the conventional subcutaneous/intramuscular route, even when the same small dose was given (*13*).

Nature of Endemic H2N2 Postpandemic Infection

The Asian influenza experience provided the first opportunity to study how the postpandemic infection and disease into an endemic phase subsided. In studies conducted in separate and disparate populations (*14*), the populations compared were Navajo school children and New York City medical students. In both groups, subclinical infections occurred each year during the 3-year study period, and clinically manifested infections decreased in conjunction with an increasing level of H2N2-specific hemagglutination inhibition antibody.

A decreasing incidence of clinically manifested cases can be ascribed either to the increase in antibody levels in the community or to a change in the intrinsic virulence of

the virus. Therefore, the nature of the disease during the endemic period is important to define. A study (*15*) in 1960 of hospitalized patients with laboratory-confirmed infections demonstrated a spectrum of disease from uncomplicated 3-day illnesses to fatal pneumonia, all in the absence of discernible epidemic influenza in the community (*15*). Asian (H2N2) virus was destined for short survival in the human population and disappeared only 11 years after its arrival. It was supplanted by the Hong Kong (H3N2) subtype.

1968: Hong Kong Influenza (H3N2)

As in 1957, a new influenza pandemic arose in Southeast Asia and acquired the sobriquet Hong Kong influenza on the basis of the site of its emergence to western attention. Once again, the daily press sounded the alarm with a brief report of a large Hong Kong epidemic in the Times of London. A decade after the 1957 pandemic, epidemiologic communication with mainland China was even less efficient than it had been earlier.

As this epidemic progressed, initially throughout Asia, important differences in the pattern of illness and death were noted. In Japan, epidemics were small, scattered, and desultory until the end of 1968. Most striking was the high illness and death rates in the United States following introduction of the virus on the West Coast. This experience stood in contrast with the experience in western Europe, including the United Kingdom, in which increased illness occurred in the absence of increased death rates in 1968–1969 and increased death rates were not seen until the following year of the pandemic.

Since the Hong Kong virus differed from its antecedent Asian virus by its HA antigen, but had retained the same (N2) NA antigen (*16*), researchers speculated that its more sporadic and variable impact in different regions of the world were mediated by differences in prior N2 immunity (*16–19*). Therefore, the 1968 pandemic has been aptly characterized as "smoldering" (*19*). Further evidence for the capacity of previous N2 experience to moderate the challenge of the Hong Kong virus was provided by Eickhoff and Meiklejohn (*20*), who showed that vaccination of Air Force cadets with an H2N2 adjuvant vaccine reduced subsequent influenza from verified H3N2 virus infection by 54%.

The amelioration of H3N2 virus infection by NA immunity alone is all the more remarkable because of the capacity of the virus to kill, as occurred in 1918 and 1957, although a broader spectrum of disease severity was apparent in 1968 than in 1957 (*15*). Although not necessarily an indication of virulence, cross-species transmission of the virus was observed (*21*). Thirty-seven years later, the H3N2 subtype still reigns as the major and most troublesome influenza A virus in humans.

Pseudopandemics and an Abortive Pandemic

Extreme Intrasubtypic Antigenic Variation and the Pseudopandemic of 1947 (H1N1)

In late 1946, an outbreak of influenza occurred in Japan and Korea in American troops. It spread in 1947 to other military bases in the United States, including Fort Monmouth, New Jersey, where the prototype FM-1 strain was isolated. The epidemic was notable because of the initial difficulty in establishing its cause as an influenza A virus because of its considerable antigenic difference from previous influenza A viruses. Indeed, for a time it was identified as "influenza A prime" (*22*). The 1947 epidemic has been thought of as a mild pandemic because the disease, although globally distributed, caused relatively few deaths. However, as a medical officer at Fort Monmouth, I can personally attest that there was nothing mild about the illness in young recruits in whom signs and symptoms closely matched those of earlier descriptions of influenza (*23*).

Most remarkable was the total failure of vaccine containing a 1943 H1N1 strain (effective in the 1943–1944 and 1944–1945 seasons) to protect the large number of US military personnel who were vaccinated. Previously, antigenic variation had been noted, but never had it been of a sufficient degree to compromise vaccine-induced immunity (24). Years later, extensive characterization of HA and NA antigens of the 1943 and 1947 viruses and comparison of their nucleotide and amino acid sequences showed marked differences in the viruses isolated in these 2 years; studies in a mouse model also showed that the 1943 vaccine afforded no protection to the 1947 virus challenge (*24*). Studies in the Fort Monmouth epidemic also documented, by serial bacterial cultures, for the first time the long suspected relationship of influenza to group A streptococcal carriage and disease (*23*).

1976: Abortive, Potentially Pandemic, Swine Influenza Virus Epidemic, Fort Dix, New Jersey (H1N1)

In the interest of full disclosure, I predicted the possibility of an imminent pandemic in an op ed piece published in The New York Times on February 13, 1976 (*25*). On February 13, I was notified that influenza viruses isolated from patients at Fort Dix, New Jersey, a few days earlier and provisionally identified as swine influenza viruses were being mailed to my laboratory in New York City. A high-yield (6:2) genetic reassortant virus (X-53) was produced and later used as a vaccine in a clinical trial in 3,000 people. An even higher yielding HA mutant virus, X-53a, was selected from X-53 and subsequently used in the mass vaccination of 43,000,000 people. (I was a member of a Center for Disease Control advisory committee and an ad hoc advisory committee to President Gerald Ford on

actions to be taken to protect the American public against swine influenza.) When no cases were found outside Fort Dix in subsequent months and the neurologic complication of Guillain-Barré syndrome occurred in association with administration of swine influenza vaccine, the National Immunization Program was abandoned, and the entire effort was assailed as a fiasco and disaster.

I wish only to note here that my unyielding position on the need for vaccine production and immediate vaccination (not stockpiling) had its basis in what science could be brought to bear in an unprecedented situation. This was the cocirculation in crowded recruit barracks of 2 influenza A viruses of different subtypes: H3N2, the major epidemic virus, and H1(swine) N1. The latter virus, which caused a minor (buried) epidemic and was shown to be serially transmissible in humans, was the putative virus of 1918. Would genetic reassortment of the viruses produce a monster, as is now feared with the current avian virus threat, or did interference by the far more prevalent virus H3N2 suppress further transmission of the swine virus?

Experience had shown a decrease or even disappearance of epidemic viruses in the summer. However, they return in winter to produce disease in conditions favoring transmission: indoor crowding and decreased relative humidity. None of these facts was noted by critics of the program.

1977: Russian Flu, a Juvenile, Age-restricted Pandemic, and the Return of Human H1N1 Virus

Our obsession with geographic eponyms for a disease of worldwide distribution is best illustrated by Russian, or later red influenza or red flu, which first came to attention in November 1977, in the Soviet Union. However, it was later reported as having first occurred in northeastern China in May of that year (*26*). It quickly became apparent that this rapidly spreading epidemic was almost entirely restricted to persons <25 years of age and that, in general, the disease was mild, although characterized by typical symptoms of influenza. The age distribution was attributed to the absence of H1N1 viruses in humans after 1957 and the subsequent successive dominance of the H2N2 and then the H3N2 subtypes.

When antigenic and molecular characterization of this virus showed that both the HA and NA antigens were remarkably similar to those of the 1950s, this finding had profound implications. Where had the virus been that it was relatively unchanged after 20 years? If serially (and cryptically) transmitted in humans, antigenic drift should have led to many changes after 2 decades. Reactivation of a long dormant infection was a possibility, but the idea conflicts with all we know of the biology of the virus in which a latent phase has not been found. Had the virus been in a deep freeze? This was a disturbing thought because it implied concealed experimentation with live virus, perhaps in a vaccine. Delayed mutation and consequent evolutionary stasis in an animal host are not unreasonable, but in what host? And if a full-blown epidemic did originate, it would be the first to do so in the history of modern virology, and a situation quite unlike the contemporary situation with H5N1 and its protracted epizootic phase. Thus, the final answer to the 1977 epidemic is not yet known.

Influenza Pandemics of the 21th Century: the Murky Crystal Ball

All pandemics are different. The minimum requirement seems to be a major change or shift in the HA antigen (1968). In 1957, changes in both HA and NA antigens were associated with higher rates of illness and death. The memorable and probably unique severity of the 1918 pandemic may have depended, at least in part, on wartime conditions and secondary bacterial infections in the absence of antimicrobial drugs. Also, mechanical respirators and supplemental oxygen were not available. Although evidence is strong that recombinational capture of animal influenza HA or NA antigens may be essential for pandemic origins, extreme antigenic drift, such as that which occurred in 1947 (*24*), can lead to global dissemination and disease by the multiply mutated virus.

An intrasubtypic H1N1 animal variant virus (A/H1N1/swine) caused serially transmitted disease, pneumonia, and death at a military installation, yet disappeared within a few weeks (1976, Fort Dix). However, in 1977 an age-restricted pandemic was caused by the revisitation of an H1N1 virus and its ability to infect persons who had not experienced the virus earlier.

Within the brief period of modern virology, of the 16 HA subtypes known to exist, pandemics have been caused only by viruses of the H1, H2, and H3 subtypes. Moreover, serologic and epidemiologic evidence has shown that each of these subtypes has produced pandemics in the past. Are these the default human subtypes? If so, can we be less concerned about the threat of contemporary epizootics?

Preparing for the Unpredictable

Yes, we can prepare, but with the realization that no amount of hand washing, hand wringing, public education, or gauze masks will do the trick (*27*). The keystone of influenza prevention is vaccination. It is unreasonable to believe that we can count on prophylaxis with antiviral agents to protect a large, vulnerable population for more than a few days at a time, and that is not long enough. How long will they be given? To whom? What are the risks in mass administration? All of this is unknown.

But vaccination against what? We do not know. Perhaps against H5N1. But do we not already have a vaccine? No,

we do not; no vaccine of adequate antigenic potency is available in sufficient supply.

The answer lies in an approach first suggested at a World Health Organization meeting in 1969 (*28*) and repeatedly endorsed since by virtually every pandemic preparedness planning group. This recommendation assumes that the nature of the next pandemic virus cannot be predicted, but that it will arise from 1 of the 16 known HA subtypes in avian or mammalian species. Accordingly, preparation by genetic reassortment of high-yield seed viruses of all HA subtypes should proceed as soon as possible for potential use in vaccine production (*28*). Thirty-seven years later, this goal has not yet been achieved. Reassortant viruses have been used in vaccine production since 1971 in response to the emergence of antigenic drift variants. A repository at the National Institute of Allergy and Infectious Diseases (www.flu-archive.org) contains recently made early and late H2N2 candidate vaccine reassortant viruses that could address the return of that virus subtype, a high-yield H7N7 reassortant virus, and a high-yield H5N3 wt mutant that does not kill either chickens or fertile hen eggs (E.D. Kilbourne, M. Perdue, unpub. data). Recently, a high-growth vaccine strain has also been developed as a pandemic vaccine candidate for protection against the threat of H9N2 virus (*29*) by what has become the standard technique of reassortment with A/PR/8/34 (H1N1) virus (*28*).

One concern about previous and anticipatory preparation and characterization of high-yield reassortants is that they may not exactly match the newly emerging strain of that subtype. Perhaps not, but in the face of a pandemic threat they could serve as barricade vaccines (*27*), ready to be pulled out of the freezer at the first threat from any subtype.

Postscript

Back to Reality: Urgent Questions That Can and Should be Answered Immediately

In assessing pandemic risk, we seem to have forgotten that influenza virus contains not 1, but 2 immunogenic protective antigens. As a case in point, I am not satisfied that we have sufficiently examined immunity to the N1 antigen of the H5N1 pandemic-candidate virus. Did infected persons who died lack antibody to N1 in their acute-phase sera? To what extent, if any, do the N1 antigens of human strains crossreact with those of the H5N1 variants? Is the antibody response to N1 antigen being examined in recipients in recent H5N1 virus vaccine trials? Mindful of the damping effect of N2 antibody in the 1968 pandemic, we might find reassurance and explanations in learning these results.

Dr Kilbourne, emeritus professor of microbiology and immunology at New York Medical College, has spent his professional life in the study of infectious diseases, particularly virus infections. His early studies of coxsackieviruses and herpes simplex preceded study of influenza in all of its manifestations. Primary contributions have been to understanding of influenza virus structure, genetics, molecular epidemiology, and pathogenesis. His studies of influenza virus genetics resulted in the first genetically engineered vaccine for the prevention of human disease, and a new approach to influenza immunization received 2 US patents.

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Address for correspondence: Edwin D. Kilbourne, 23 Willard Ave, Madison, CT 06443, USA; fax: 203-318-0036; email: ekilbourne@snet.net

1918 Influenza: the Mother of All Pandemics

Jeffery K. Taubenberger* and David M. Morens†

The "Spanish" influenza pandemic of 1918–1919, which caused ≈50 million deaths worldwide, remains an ominous warning to public health. Many questions about its origins, its unusual epidemiologic features, and the basis of its pathogenicity remain unanswered. The public health implications of the pandemic therefore remain in doubt even as we now grapple with the feared emergence of a pandemic caused by H5N1 or other virus. However, new information about the 1918 virus is emerging, for example, sequencing of the entire genome from archival autopsy tissues. But, the viral genome alone is unlikely to provide answers to some critical questions. Understanding the 1918 pandemic and its implications for future pandemics requires careful experimentation and in-depth historical analysis.

"Curiouser and curiouser!" cried Alice

Lewis Carroll, Alice's Adventures in Wonderland, 1865

An estimated one third of the world's population (or ≈500 million persons) were infected and had clinically apparent illnesses (*1*,*2*) during the 1918–1919 influenza pandemic. The disease was exceptionally severe. Case-fatality rates were >2.5%, compared to <0.1% in other influenza pandemics (*3*,*4*). Total deaths were estimated at ≈50 million (*5*–*7*) and were arguably as high as 100 million (*7*).

The impact of this pandemic was not limited to 1918–1919. All influenza A pandemics since that time, and indeed almost all cases of influenza A worldwide (excepting human infections from avian viruses such as H5N1 and H7N7), have been caused by descendants of the 1918 virus, including "drifted" H1N1 viruses and reassorted H2N2 and H3N2 viruses. The latter are composed of key genes from the 1918 virus, updated by subsequently-incorporated avian influenza genes that code for novel surface proteins, making the 1918 virus indeed the "mother" of all pandemics.

In 1918, the cause of human influenza and its links to avian and swine influenza were unknown. Despite clinical and epidemiologic similarities to influenza pandemics of 1889, 1847, and even earlier, many questioned whether such an explosively fatal disease could be influenza at all. That question did not begin to be resolved until the 1930s, when closely related influenza viruses (now known to be H1N1 viruses) were isolated, first from pigs and shortly thereafter from humans. Seroepidemiologic studies soon linked both of these viruses to the 1918 pandemic (*8*). Subsequent research indicates that descendants of the 1918 virus still persists enzootically in pigs. They probably also circulated continuously in humans, undergoing gradual antigenic drift and causing annual epidemics, until the 1950s. With the appearance of a new H2N2 pandemic strain in 1957 ("Asian flu"), the direct H1N1 viral descendants of the 1918 pandemic strain disappeared from human circulation entirely, although the related lineage persisted enzootically in pigs. But in 1977, human H1N1 viruses suddenly "reemerged" from a laboratory freezer (*9*). They continue to circulate endemically and epidemically.

Thus in 2006, 2 major descendant lineages of the 1918 H1N1 virus, as well as 2 additional reassortant lineages, persist naturally: a human epidemic/endemic H1N1 lineage, a porcine enzootic H1N1 lineage (so-called classic swine flu), and the reassorted human H3N2 virus lineage, which like the human H1N1 virus, has led to a porcine H3N2 lineage. None of these viral descendants, however, approaches the pathogenicity of the 1918 parent virus. Apparently, the porcine H1N1 and H3N2 lineages uncommonly infect humans, and the human H1N1 and H3N2 lineages have both been associated with substantially lower rates of illness and death than the virus of 1918. In fact, current H1N1 death rates are even lower than those for H3N2 lineage strains (prevalent from 1968 until the present). H1N1 viruses descended from the 1918 strain, as well as

*Armed Forces Institute of Pathology, Rockville, Maryland, USA; and †National Institutes of Health, Bethesda, Maryland, USA

H3N2 viruses, have now been cocirculating worldwide for 29 years and show little evidence of imminent extinction.

Trying To Understand What Happened

By the early 1990s, 75 years of research had failed to answer a most basic question about the 1918 pandemic: why was it so fatal? No virus from 1918 had been isolated, but all of its apparent descendants caused substantially milder human disease. Moreover, examination of mortality data from the 1920s suggests that within a few years after 1918, influenza epidemics had settled into a pattern of annual epidemicity associated with strain drifting and substantially lowered death rates. Did some critical viral genetic event produce a 1918 virus of remarkable pathogenicity and then another critical genetic event occur soon after the 1918 pandemic to produce an attenuated H1N1 virus?

In 1995, a scientific team identified archival influenza autopsy materials collected in the autumn of 1918 and began the slow process of sequencing small viral RNA fragments to determine the genomic structure of the causative influenza virus (*10*). These efforts have now determined the complete genomic sequence of 1 virus and partial sequences from 4 others. The primary data from the above studies (*11–17*) and a number of reviews covering different aspects of the 1918 pandemic have recently been published (*18–20*) and confirm that the 1918 virus is the likely ancestor of all 4 of the human and swine H1N1 and H3N2 lineages, as well as the "extinct" H2N2 lineage. No known mutations correlated with high pathogenicity in other human or animal influenza viruses have been found in the 1918 genome, but ongoing studies to map virulence factors are yielding interesting results. The 1918 sequence data, however, leave unanswered questions about the origin of the virus (*19*) and about the epidemiology of the pandemic.

When and Where Did the 1918 Influenza Pandemic Arise?

Before and after 1918, most influenza pandemics developed in Asia and spread from there to the rest of the world. Confounding definite assignment of a geographic point of origin, the 1918 pandemic spread more or less simultaneously in 3 distinct waves during an ≈12-month period in 1918–1919, in Europe, Asia, and North America (the first wave was best described in the United States in March 1918). Historical and epidemiologic data are inadequate to identify the geographic origin of the virus (*21*), and recent phylogenetic analysis of the 1918 viral genome does not place the virus in any geographic context (*19*).

Although in 1918 influenza was not a nationally reportable disease and diagnostic criteria for influenza and pneumonia were vague, death rates from influenza and pneumonia in the United States had risen sharply in 1915 and 1916 because of a major respiratory disease epidemic beginning in December 1915 (*22*). Death rates then dipped slightly in 1917. The first pandemic influenza wave appeared in the spring of 1918, followed in rapid succession by much more fatal second and third waves in the fall and winter of 1918–1919, respectively (Figure 1). Is it possible that a poorly-adapted H1N1 virus was already beginning to spread in 1915, causing some serious illnesses but not yet sufficiently fit to initiate a pandemic? Data consistent with this possibility were reported at the time from European military camps (*23*), but a counter argument is that if a strain with a new hemagglutinin (HA) was causing enough illness to affect the US national death rates from pneumonia and influenza, it should have caused a pandemic sooner, and when it eventually did, in 1918, many people should have been immune or at least partially immunoprotected. "Herald" events in 1915, 1916, and possibly even in early 1918, if they occurred, would be difficult to identify.

The 1918 influenza pandemic had another unique feature, the simultaneous (or nearly simultaneous) infection of humans and swine. The virus of the 1918 pandemic likely expressed an antigenically novel subtype to which most humans and swine were immunologically naive in 1918 (*12*,*20*). Recently published sequence and phylogenetic analyses suggest that the genes encoding the HA and neuraminidase (NA) surface proteins of the 1918 virus were derived from an avianlike influenza virus shortly before the start of the pandemic and that the precursor virus had not circulated widely in humans or swine in the few decades before (*12*,*15*,*24*). More recent analyses of the other gene segments of the virus also support this conclusion. Regression analyses of human and swine influenza sequences obtained from 1930 to the present place the initial circulation of the 1918 precursor virus in humans at approximately 1915–1918 (*20*). Thus, the precursor was probably not circulating widely in humans until shortly before 1918, nor did it appear to have jumped directly from any species of bird studied to date (*19*). In summary, its origin remains puzzling.

Were the 3 Waves in 1918–1919 Caused by the Same Virus? If So, How and Why?

Historical records since the 16th century suggest that new influenza pandemics may appear at any time of year, not necessarily in the familiar annual winter patterns of interpandemic years, presumably because newly shifted influenza viruses behave differently when they find a universal or highly susceptible human population. Thereafter, confronted by the selection pressures of population immunity, these pandemic viruses begin to drift genetically and eventually settle into a pattern of annual epidemic recurrences caused by the drifted virus variants.

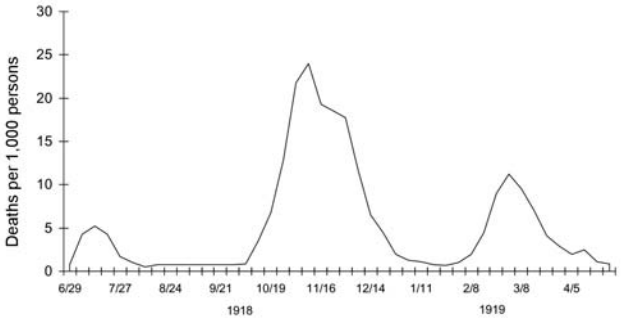

Figure 1. Three pandemic waves: weekly combined influenza and pneumonia mortality, United Kingdom, 1918–1919 (*21*).

In the 1918–1919 pandemic, a first or spring wave began in March 1918 and spread unevenly through the United States, Europe, and possibly Asia over the next 6 months (Figure 1). Illness rates were high, but death rates in most locales were not appreciably above normal. A second or fall wave spread globally from September to November 1918 and was highly fatal. In many nations, a third wave occurred in early 1919 (*21*). Clinical similarities led contemporary observers to conclude initially that they were observing the same disease in the successive waves. The milder forms of illness in all 3 waves were identical and typical of influenza seen in the 1889 pandemic and in prior interpandemic years. In retrospect, even the rapid progressions from uncomplicated influenza infections to fatal pneumonia, a hallmark of the 1918–1919 fall and winter waves, had been noted in the relatively few severe spring wave cases. The differences between the waves thus seemed to be primarily in the much higher frequency of complicated, severe, and fatal cases in the last 2 waves.

But 3 extensive pandemic waves of influenza within 1 year, occurring in rapid succession, with only the briefest of quiescent intervals between them, was unprecedented. The occurrence, and to some extent the severity, of recurrent annual outbreaks, are driven by viral antigenic drift, with an antigenic variant virus emerging to become dominant approximately every 2 to 3 years. Without such drift, circulating human influenza viruses would presumably disappear once herd immunity had reached a critical threshold at which further virus spread was sufficiently limited. The timing and spacing of influenza epidemics in interpandemic years have been subjects of speculation for decades. Factors believed to be responsible include partial herd immunity limiting virus spread in all but the most favorable circumstances, which include lower environmental temperatures and human nasal temperatures (beneficial to thermolabile viruses such as influenza), optimal humidity, increased crowding indoors, and imperfect ventilation due to closed windows and suboptimal airflow.

However, such factors cannot explain the 3 pandemic waves of 1918–1919, which occurred in the spring-summer, summer-fall, and winter (of the Northern Hemisphere), respectively. The first 2 waves occurred at a time of year normally unfavorable to influenza virus spread. The second wave caused simultaneous outbreaks in the Northern and Southern Hemispheres from September to November. Furthermore, the interwave periods were so brief as to be almost undetectable in some locales. Reconciling epidemiologically the steep drop in cases in the first and second waves with the sharp rises in cases of the second and third waves is difficult. Assuming even transient postinfection immunity, how could susceptible persons be too few to sustain transmission at 1 point, and yet enough to start a new explosive pandemic wave a few weeks later? Could the virus have mutated profoundly and almost simultaneously around the world, in the short periods between the successive waves? Acquiring viral drift sufficient to produce new influenza strains capable of escaping population immunity is believed to take years of global circulation, not weeks of local circulation. And having occurred, such mutated viruses normally take months to spread around the world.

At the beginning of other "off season" influenza pandemics, successive distinct waves within a year have not been reported. The 1889 pandemic, for example, began in the late spring of 1889 and took several months to spread throughout the world, peaking in northern Europe and the United States late in 1889 or early in 1890. The second recurrence peaked in late spring 1891 (more than a year after the first pandemic appearance) and the third in early 1892 (*21*). As was true for the 1918 pandemic, the second 1891 recurrence produced of the most deaths. The 3 recurrences in 1889–1892, however, were spread over >3 years, in contrast to 1918–1919, when the sequential waves seen in individual countries were typically compressed into ≈8–9 months.

What gave the 1918 virus the unprecedented ability to generate rapidly successive pandemic waves is unclear. Because the only 1918 pandemic virus samples we have yet identified are from second-wave patients (*16*), nothing can yet be said about whether the first (spring) wave, or for that matter, the third wave, represented circulation of the same virus or variants of it. Data from 1918 suggest that persons infected in the second wave may have been protected from influenza in the third wave. But the few data bearing on protection during the second and third waves after infection in the first wave are inconclusive and do little to resolve the question of whether the first wave was caused by the same virus or whether major genetic evolutionary events were occurring even as the pandemic

exploded and progressed. Only influenza RNA–positive human samples from before 1918, and from all 3 waves, can answer this question.

What Was the Animal Host Origin of the Pandemic Virus?

Viral sequence data now suggest that the entire 1918 virus was novel to humans in, or shortly before, 1918, and that it thus was not a reassortant virus produced from old existing strains that acquired 1 or more new genes, such as those causing the 1957 and 1968 pandemics. On the contrary, the 1918 virus appears to be an avianlike influenza virus derived in toto from an unknown source (*17,19*), as its 8 genome segments are substantially different from contemporary avian influenza genes. Influenza virus gene sequences from a number of fixed specimens of wild birds collected circa 1918 show little difference from avian viruses isolated today, indicating that avian viruses likely undergo little antigenic change in their natural hosts even over long periods (*24,25*).

For example, the 1918 nucleoprotein (NP) gene sequence is similar to that of viruses found in wild birds at the amino acid level but very divergent at the nucleotide level, which suggests considerable evolutionary distance between the sources of the 1918 NP and of currently sequenced NP genes in wild bird strains (*13,19*). One way of looking at the evolutionary distance of genes is to compare ratios of synonymous to nonsynonymous nucleotide substitutions. A synonymous substitution represents a silent change, a nucleotide change in a codon that does not result in an amino acid replacement. A nonsynonymous substitution is a nucleotide change in a codon that results in an amino acid replacement. Generally, a viral gene subjected to immunologic drift pressure or adapting to a new host exhibits a greater percentage of nonsynonymous mutations, while a virus under little selective pressure accumulates mainly synonymous changes. Since little or no selection pressure is exerted on synonymous changes, they are thought to reflect evolutionary distance.

Because the 1918 gene segments have more synonymous changes from known sequences of wild bird strains than expected, they are unlikely to have emerged directly from an avian influenza virus similar to those that have been sequenced so far. This is especially apparent when one examines the differences at 4-fold degenerate codons, the subset of synonymous changes in which, at the third codon position, any of the 4 possible nucleotides can be substituted without changing the resulting amino acid. At the same time, the 1918 sequences have too few amino acid differences from those of wild-bird strains to have spent many years adapting only in a human or swine intermediate host. One possible explanation is that these unusual gene segments were acquired from a reservoir of influenza virus that has not yet been identified or sampled. All of these findings beg the question: where did the 1918 virus come from?

In contrast to the genetic makeup of the 1918 pandemic virus, the novel gene segments of the reassorted 1957 and 1968 pandemic viruses all originated in Eurasian avian viruses (*26*); both human viruses arose by the same mechanism—reassortment of a Eurasian wild waterfowl strain with the previously circulating human H1N1 strain. Proving the hypothesis that the virus responsible for the 1918 pandemic had a markedly different origin requires samples of human influenza strains circulating before 1918 and samples of influenza strains in the wild that more closely resemble the 1918 sequences.

What Was the Biological Basis for 1918 Pandemic Virus Pathogenicity?

Sequence analysis alone does not offer clues to the pathogenicity of the 1918 virus. A series of experiments are under way to model virulence in vitro and in animal models by using viral constructs containing 1918 genes produced by reverse genetics.

Influenza virus infection requires binding of the HA protein to sialic acid receptors on host cell surface. The HA receptor-binding site configuration is different for those influenza viruses adapted to infect birds and those adapted to infect humans. Influenza virus strains adapted to birds preferentially bind sialic acid receptors with α (2–3) linked sugars (*27–29*). Human-adapted influenza viruses are thought to preferentially bind receptors with α (2–6) linkages. The switch from this avian receptor configuration requires of the virus only 1 amino acid change (*30*), and the HAs of all 5 sequenced 1918 viruses have this change, which suggests that it could be a critical step in human host adaptation. A second change that greatly augments virus binding to the human receptor may also occur, but only 3 of 5 1918 HA sequences have it (*16*).

This means that at least 2 H1N1 receptor-binding variants cocirculated in 1918: 1 with high-affinity binding to the human receptor and 1 with mixed-affinity binding to both avian and human receptors. No geographic or chronologic indication exists to suggest that one of these variants was the precursor of the other, nor are there consistent differences between the case histories or histopathologic features of the 5 patients infected with them. Whether the viruses were equally transmissible in 1918, whether they had identical patterns of replication in the respiratory tree, and whether one or both also circulated in the first and third pandemic waves, are unknown.

In a series of in vivo experiments, recombinant influenza viruses containing between 1 and 5 gene segments of the 1918 virus have been produced. Those constructs bearing the 1918 HA and NA are all highly pathogenic in

mice (*31*). Furthermore, expression microarray analysis performed on whole lung tissue of mice infected with the 1918 HA/NA recombinant showed increased upregulation of genes involved in apoptosis, tissue injury, and oxidative damage (*32*). These findings are unexpected because the viruses with the 1918 genes had not been adapted to mice; control experiments in which mice were infected with modern human viruses showed little disease and limited viral replication. The lungs of animals infected with the 1918 HA/NA construct showed bronchial and alveolar epithelial necrosis and a marked inflammatory infiltrate, which suggests that the 1918 HA (and possibly the NA) contain virulence factors for mice. The viral genotypic basis of this pathogenicity is not yet mapped. Whether pathogenicity in mice effectively models pathogenicity in humans is unclear. The potential role of the other 1918 proteins, singularly and in combination, is also unknown. Experiments to map further the genetic basis of virulence of the 1918 virus in various animal models are planned. These experiments may help define the viral component to the unusual pathogenicity of the 1918 virus but cannot address whether specific host factors in 1918 accounted for unique influenza mortality patterns.

Why Did the 1918 Virus Kill So Many Healthy Young Adults?

The curve of influenza deaths by age at death has historically, for at least 150 years, been U-shaped (Figure 2), exhibiting mortality peaks in the very young and the very old, with a comparatively low frequency of deaths at all ages in between. In contrast, age-specific death rates in the 1918 pandemic exhibited a distinct pattern that has not been documented before or since: a "W-shaped" curve, similar to the familiar U-shaped curve but with the addition of a third (middle) distinct peak of deaths in young adults ≈20–40 years of age. Influenza and pneumonia death rates for those 15–34 years of age in 1918–1919, for example, were >20 times higher than in previous years (*35*). Overall, nearly half of the influenza-related deaths in the 1918 pandemic were in young adults 20–40 years of age, a phenomenon unique to that pandemic year. The 1918 pandemic is also unique among influenza pandemics in that absolute risk of influenza death was higher in those <65 years of age than in those >65; persons <65 years of age accounted for >99% of all excess influenza-related deaths in 1918–1919. In comparison, the <65-year age group accounted for 36% of all excess influenza-related deaths in the 1957 H2N2 pandemic and 48% in the 1968 H3N2 pandemic (33).

A sharper perspective emerges when 1918 age-specific influenza morbidity rates (21) are used to adjust the W-shaped mortality curve (Figure 3, panels, A, B, and C [*35*,*37*]). Persons <35 years of age in 1918 had a disproportionately high influenza incidence (Figure 3, panel A). But even after adjusting age-specific deaths by age-specific clinical attack rates (Figure 3, panel B), a W-shaped curve with a case-fatality peak in young adults remains and is significantly different from U-shaped age-specific case-fatality curves typically seen in other influenza years, e.g., 1928–1929 (Figure 3, panel C). Also, in 1918 those 5 to 14 years of age accounted for a disproportionate number of influenza cases, but had a much lower death rate from influenza and pneumonia than other age groups. To explain this pattern, we must look beyond properties of the virus to host and environmental factors, possibly including immunopathology (e.g., antibody-dependent infection enhancement associated with prior virus exposures [*38*]) and exposure to risk cofactors such as coinfecting agents, medications, and environmental agents.

One theory that may partially explain these findings is that the 1918 virus had an intrinsically high virulence, tempered only in those patients who had been born before 1889, e.g., because of exposure to a then-circulating virus capable of providing partial immunoprotection against the 1918 virus strain only in persons old enough (>35 years) to have been infected during that prior era (*35*). But this theory would present an additional paradox: an obscure precursor virus that left no detectable trace today would have had to have appeared and disappeared before 1889 and then reappeared more than 3 decades later.

Epidemiologic data on rates of clinical influenza by age, collected between 1900 and 1918, provide good evidence for the emergence of an antigenically novel influenza virus in 1918 (*21*). Jordan showed that from 1900 to 1917, the 5- to 15-year age group accounted for 11% of total influenza cases, while the >65-year age group accounted for 6 % of influenza cases. But in 1918, cases in

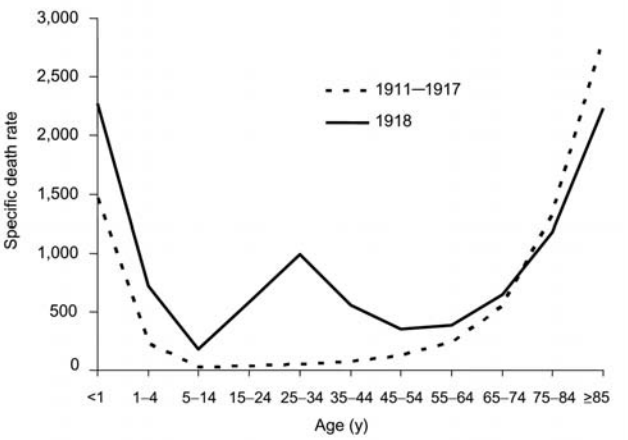

Figure 2. "U-" and "W-" shaped combined influenza and pneumonia mortality, by age at death, per 100,000 persons in each age group, United States, 1911–1918. Influenza- and pneumonia-specific death rates are plotted for the interpandemic years 1911–1917 (dashed line) and for the pandemic year 1918 (solid line) (*33*,*34*).

Figure 3. Influenza plus pneumonia (P&I) (combined) age-specific incidence rates per 1,000 persons per age group (panel A), death rates per 1,000 persons, ill and well combined (panel B), and case-fatality rates (panel C, solid line), US Public Health Service house-to-house surveys, 8 states, 1918 (*36*). A more typical curve of age-specific influenza case-fatality (panel C, dotted line) is taken from US Public Health Service surveys during 1928–1929 (*37*).

the 5 to 15-year-old group jumped to 25% of influenza cases (compatible with exposure to an antigenically novel virus strain), while the >65-year age group only accounted for 0.6% of the influenza cases, findings consistent with previously acquired protective immunity caused by an identical or closely related viral protein to which older persons had once been exposed. Mortality data are in accord. In 1918, persons >75 years had lower influenza and pneumonia case-fatality rates than they had during the prepandemic period of 1911–1917. At the other end of the age spectrum (Figure 2), a high proportion of deaths in infancy and early childhood in 1918 mimics the age pattern, if not the mortality rate, of other influenza pandemics.

Could a 1918-like Pandemic Appear Again? If So, What Could We Do About It?

In its disease course and pathologic features, the 1918 pandemic was different in degree, but not in kind, from previous and subsequent pandemics. Despite the extraordinary number of global deaths, most influenza cases in 1918 (>95% in most locales in industrialized nations) were mild and essentially indistinguishable from influenza cases today. Furthermore, laboratory experiments with recombinant influenza viruses containing genes from the 1918 virus suggest that the 1918 and 1918-like viruses would be as sensitive as other typical virus strains to the Food and Drug Administration–approved antiinfluenza drugs rimantadine and oseltamivir.

However, some characteristics of the 1918 pandemic appear unique: most notably, death rates were 5 – 20 times higher than expected. Clinically and pathologically, these high death rates appear to be the result of several factors, including a higher proportion of severe and complicated infections of the respiratory tract, rather than involvement of organ systems outside the normal range of the influenza virus. Also, the deaths were concentrated in an unusually young age group. Finally, in 1918, 3 separate recurrences of influenza followed each other with unusual rapidity, resulting in 3 explosive pandemic waves within a year's time (Figure 1). Each of these unique characteristics may reflect genetic features of the 1918 virus, but understanding them will also require examination of host and environmental factors.

Until we can ascertain which of these factors gave rise to the mortality patterns observed and learn more about the formation of the pandemic, predictions are only educated guesses. We can only conclude that since it happened once, analogous conditions could lead to an equally devastating pandemic.

Like the 1918 virus, H5N1 is an avian virus (*39*), though a distantly related one. The evolutionary path that led to pandemic emergence in 1918 is entirely unknown, but it appears to be different in many respects from the current situation with H5N1. There are no historical data, either in 1918 or in any other pandemic, for establishing that a pandemic "precursor" virus caused a highly pathogenic outbreak in domestic poultry, and no highly pathogenic avian influenza (HPAI) virus, including H5N1 and a number of others, has ever been known to cause a major human epidemic, let alone a pandemic. While data bearing on influenza virus human cell adaptation (e.g., receptor

binding) are beginning to be understood at the molecular level, the basis for viral adaptation to efficient human-to-human spread, the chief prerequisite for pandemic emergence, is unknown for any influenza virus. The 1918 virus acquired this trait, but we do not know how, and we currently have no way of knowing whether H5N1 viruses are now in a parallel process of acquiring human-to-human transmissibility. Despite an explosion of data on the 1918 virus during the past decade, we are not much closer to understanding pandemic emergence in 2006 than we were in understanding the risk of H1N1 "swine flu" emergence in 1976.

Even with modern antiviral and antibacterial drugs, vaccines, and prevention knowledge, the return of a pandemic virus equivalent in pathogenicity to the virus of 1918 would likely kill >100 million people worldwide. A pandemic virus with the (alleged) pathogenic potential of some recent H5N1 outbreaks could cause substantially more deaths.

Whether because of viral, host or environmental factors, the 1918 virus causing the first or 'spring' wave was not associated with the exceptional pathogenicity of the second (fall) and third (winter) waves. Identification of an influenza RNA-positive case from the first wave could point to a genetic basis for virulence by allowing differences in viral sequences to be highlighted. Identification of pre-1918 human influenza RNA samples would help us understand the timing of emergence of the 1918 virus. Surveillance and genomic sequencing of large numbers of animal influenza viruses will help us understand the genetic basis of host adaptation and the extent of the natural reservoir of influenza viruses. Understanding influenza pandemics in general requires understanding the 1918 pandemic in all its historical, epidemiologic, and biologic aspects.

Dr Taubenberger is chair of the Department of Molecular Pathology at the Armed Forces Institute of Pathology, Rockville, Maryland. His research interests include the molecular pathophysiology and evolution of influenza viruses.

Dr Morens is an epidemiologist with a long-standing interest in emerging infectious diseases, virology, tropical medicine, and medical history. Since 1999, he has worked at the National Institute of Allergy and Infectious Diseases.

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Address for correspondence: Jeffery K. Taubenberger, Department of Molecular Pathology, Armed Forces Institute of Pathology, 1413 Research Blvd, Bldg 101, Rm 1057, Rockville, MD 20850-3125, USA; fax. 301-295-9507; email: taubenberger@afip.osd.mil

Swine Influenza A Outbreak, Fort Dix, New Jersey, 1976

Joel C. Gaydos,* Franklin H. Top, Jr,† Richard A. Hodder,‡ and Philip K. Russell§[1]

In early 1976, the novel A/New Jersey/76 (Hsw1N1) influenza virus caused severe respiratory illness in 13 soldiers with 1 death at Fort Dix, New Jersey. Since A/New Jersey was similar to the 1918–1919 pandemic virus, rapid outbreak assessment and enhanced surveillance were initiated. A/New Jersey virus was detected only from January 19 to February 9 and did not spread beyond Fort Dix. A/Victoria/75 (H3N2) spread simultaneously, also caused illness, and persisted until March. Up to 230 soldiers were infected with the A/New Jersey virus. Rapid recognition of A/New Jersey, swift outbreak assessment, and enhanced surveillance resulted from excellent collaboration between Fort Dix, New Jersey Department of Health, Walter Reed Army Institute of Research, and Center for Disease Control personnel. Despite efforts to define the events at Fort Dix, many questions remain unanswered, including the following: Where did A/New Jersey come from? Why did transmission stop?

Revisiting events surrounding the 1976 swine influenza A (H1N1) outbreak may assist those planning for the rapid identification and characterization of threatening contemporary viruses, like avian influenza A (H5N1) (*1*). The severity of the 1918 influenza A (H1N1) pandemic and evidence for a cycle of pandemics aroused concern that the 1918 disaster could recur (*2*,*3*). Following the 1918 pandemic, H1N1 strains circulated until the "Asian" influenza A (H2N2) pandemic in 1957 (*3*). When in early 1976, cases of influenza in soldiers, mostly recruits, at Fort Dix, New Jersey, were associated with isolation of influenza A (H1N1) serotypes (which in 1976 were labeled Hsw1N1), an intense investigation followed (*4*).

Of 19,000 people at Fort Dix in January 1976, ≈32% were recruits (basic trainees) (*4*). Recruits reported to Fort Dix for 7 weeks of initial training through the basic training reception center, where they lived and were processed into the Army during an intense 3 days of examinations, administrative procedures, and indoctrination. At the reception center, training unit cohorts were formed. Recruits were grouped into 50-member units (platoons) and organized into companies of 4 platoons each. Units formed by week's end moved from the reception center to the basic training quarters. To prevent respiratory illnesses, recruits were isolated in their company areas for 2 weeks and restricted to the military post for 4 weeks (*4*). Platoon members had close contact with other platoon members, less contact with other platoons in their company, and even less contact with other companies.

On arrival, recruits received the 1975–1976 influenza vaccine (A/Port Chalmers/1/73 [H3N2], A/Scotland/840/74 [H3N2], and B/Hong Kong/15/72) (*4*). Other soldiers reported directly to advanced training programs of 4 to 12 weeks at Fort Dix immediately after basic training at Fort Dix or elsewhere. These soldiers received influenza vaccinations in basic training. Civilian employees and soldiers' families were offered vaccine, but only an estimated <40% accepted (*4*).

Training stopped over the Christmas–New Year's holidays and resumed on January 5, 1976, with an influx of new trainees. The weather was cold (wind chill factors of 0° to –43°F), and the reception center was crowded (*4*). Resumption of training was associated with an explosive febrile respiratory disease outbreak involving new arrivals and others. Throat swabs were collected from a sample of hospitalized soldiers with this syndrome. On January 23, the Fort Dix preventive medicine physician learned of 2 isolations of adenovirus type 21 and suspected an adenovirus outbreak (*4*). He notified the county health department and the New Jersey (NJ) Department of Health of the

*Walter Reed Army Institute of Research, Silver Spring, Maryland, USA; †MedImmune, Incorporated, Gaithersburg, Maryland, USA; ‡Northeast Center for Special Care, Lake Katrine, New York, USA; and §Albert B. Sabin Vaccine Institute, New Canaan, Connecticut, USA

[1]In 1976 all authors were US Army officers assigned to the Walter Reed Army Institute of Research, Washington, DC.

outbreak (*4*). On January 28, an NJ Department of Health official consulted with the military physician and suggested that the explosive, widespread outbreak could be influenza (*4*). Over the next 2 days, 19 specimens were delivered to the state laboratory and 7 A/Victoria-like viruses and 3 unknown hemagglutinating agents were identified (*4*). Specimens were flown to the Center for Disease Control (CDC), Atlanta, Georgia, on February 6, where a fourth unknown agent was found (*4*).

On February 2, Fort Dix and NJ Department of Health personnel arranged for virologic studies of deaths possibly caused by influenza (*4*). Tracheal swabs taken on February 5 from a recruit who died on February 4 yielded a fifth unknown agent on February 9. By February 10, laboratory evidence had confirmed that a novel influenza strain was circulating at Fort Dix and that 2 different influenza strains were causing disease. By February 13, all 5 unknown strains were identified as swine influenza A (Hsw1N1). The possibility of laboratory contamination was evaluated (*4*). No known swine influenza A strains were present in the NJ Department of Health Virus Laboratory before the Fort Dix outbreak. Additionally, all unknown Fort Dix viruses were independently isolated from original specimens at CDC and the Walter Reed Army Institute of Research (WRAIR), Washington, DC. Also, 2 patients with novel virus isolates had convalescent-phase, homologous, hemagglutination-inhibition (HAI) antibody titers of 1:40–1:80, consistent with recent infections. The new influenza strain had been independently identified in 3 different laboratories and supporting serologic evidence developed within 15 days after the original specimens were collected (Table) (*4*).

Swine Influenza A Viruses

The swine influenza A (Hsw1N1) viruses from Fort Dix soldiers were studied at CDC (*5*,*6*). The novel virus was named A/New Jersey/76 (Hsw1N1). Initially, HAI serologic studies of Fort Dix populations were performed at WRAIR by using inactivated A/Mayo Clinic/103/74 (Hsw1N1) antigen from CDC (*7*). The A/Mayo Clinic virus was recovered in 1974 from lung tissue obtained at autopsy from a man with Hodgkin disease who lived on a

Table. Key events in the swine influenza A (Hsw1N1) outbreak, Fort Dix, NJ

Date (1976)	Event
January 5	Outbreak of acute respiratory disease following influx of new recruit trainees (*4*).
January 19	Earliest hospitalization of Fort Dix soldier with acute respiratory disease attributed to swine influenza A (Hsw1N1) (identified retrospectively by serologic tests) (*7*,*14*)
January 21	Influenza A/Victoria (H3N2) identified away from Fort Dix in NJ civilians (*4*)
January 23	Adenovirus type 21 isolated from soldiers ill with respiratory disease: Fort Dix reported outbreak to local and state health departments (*4*)
January 28	NJ Department of Health (DoH) suggested Fort Dix outbreak may be due to influenza and offered to process specimens for virus isolation (*4*)
January 29–30	19 specimens sent to NJ Department of Health in 2 shipments (*4*)
February 2–3	NJ DoH identified 4 isolates of H3N2-like viruses and 2 unknown hemagglutinating agents in 8 specimens sent on January 29. Fort Dix and NJ DoH arranged for study of deaths possibly due to influenza. NJ DoH identified 3 H3N2-like viruses and third unknown hemagglutinating agent in 11 specimens sent on January 30 (*4*).
February 4	Fort Dix soldier died with acute respiratory disease (*4*).
February 5	Tracheal specimens from deceased soldier sent to NJ DoH (*4*).
February 6	NJ DoH sent specimens to Center for Disease Control (CDC), Atlanta, GA; CDC identified fourth unknown hemagglutinating agent in Fort Dix specimens (*4*).
February 9	Specimens from soldier who died on February 4 yielded fifth unknown hemagglutinating agent (*4*). Last hospitalization of an identified Fort Dix soldier with febrile, acute respiratory disease attributed to swine influenza A (Hsw1N1) (identified retrospectively by serologic tests) (*7*,*14*).
February 10	Laboratory evidence supported 2 influenza type A strains circulating on Fort Dix; 1 was a radically new strain. Prospective surveillance for cases in surrounding area was initiated; only cases of H3N2 were found (*4*).
February 13	Review of laboratory data and information found all 5 unknown agents were swine influenza A strains (later named A/New Jersey [Hsw1N1]); 3 laboratories independently identified swine virus from original specimens (serologic data supporting swine influenza A virus infection later obtained from 2 survivors with A/New Jersey isolates) (*4*).
February 14–16	Initial planning meeting in Atlanta, GA, between CDC, NJ DoH, Fort Dix, and Walter Reed Army Institute of Research personnel. Prospective case finding was initiated at Fort Dix; H3N2 was isolated; Hsw1N1 was not isolated (*7*). Retrospective case finding was initiated by serologic study of stored serum specimens from Fort Dix soldiers who had been hospitalized for acute respiratory disease; 8 new cases of disease due to Hsw1N1 were identified with hospitalization dates between January 19 and February 9 (*7*,*14*).
February 22–24	Prospective case finding was again conducted at Fort Dix; H3N2 virus was isolated but not Hsw1N1 (*7*).
February 27	Thirty-nine new recruits entering Fort Dix February 21–27 gave blood samples after arrival and 5 weeks later; serologic studies were consistent with influenza immunization but not spread of H3N2 virus. None had titer rise to Hsw1N1 (*11*).
March 19	Prospective surveillance identified last case of influenza in areas around Fort Dix; only H3N2 viruses were identified outside of Fort Dix (*4*).

swine farm (*8*). Later, CDC provided WRAIR with A/New Jersey/76 (Hsw1N1) antigen (*7*).

Outbreak Investigation Planning

Outbreak investigation plans were developed quickly, and lines of communication and responsibilities were defined. Since a retrospective investigation required extensive serologic studies, a serology laboratory was established at WRAIR and operated 7 days a week. The HAI antibody test, which measured antibody to the hemagglutinin glycoprotein, was used to identify infections (*9*). Variables other than 1976 swine virus infection that might influence HAI titers were identified. Influenza A (H1N1) viruses circulated from 1918 to 1957 (*3*). Additionally, earlier military influenza vaccines (1955–1969) and some civilian formulations (1956–1958) contained swine antigens (*10*). Most basic training soldiers were in their late teens and early twenties, so few had potential exposure to military vaccines (the earlier military vaccines were available to civilian workers and soldiers' families) (*10*). Other populations were expected to have age-related antibody from infections or vaccines. Development of heterotypic antibody after vaccination or infection with contemporary H3N2 antigens was possible; populations suitable for assessing this were studied. None of the potential HAI test limitations was considered serious.

The NJ Department of Health continued to provide virus isolation services to the military (*4*). Army personnel investigated the outbreak on Fort Dix; civilian health departments defined the outbreak beyond Fort Dix. CDC provided reference laboratory support and consultation.

Case Finding at Fort Dix

Case-finding was conducted prospectively and retrospectively (Table). Prospectively, throat washings were collected from patients with febrile, acute respiratory disease who were hospitalized or sought treatment at the emergency room February 14–16 (phase I, n = 50) and February 22–24 (phase II, n = 45) (*7*). Attempts were made to obtain paired serum specimens from phase I patients. Specimens were obtained from 60 basic training soldiers, 13 other military personnel, and 22 civilians. A/Victoria/75 (H3N2) virus was isolated from 34 (68%) persons during phase I and 21 (47%) in phase II (*7*). A/New Jersey/76 (Hsw1N1) was not isolated from any of the 95 patients. One of 34 (3%) persons with an A/Victoria isolate and paired serum samples had a ≥4-fold rise in titer to A/Mayo Clinic (Hsw1N1) antigen, with an acute titer of <1:10 increasing to 1:20 (*7*).

Retrospective study was made possible by an ongoing Adenovirus Surveillance Program, which collected weekly throats swabs and paired serum specimens from a sample (≈3%–6%) of basic trainees hospitalized with respiratory disease (*7*). Specimens had been sent to Army regional laboratories, and 80% of the paired serum specimens from Fort Dix trainees hospitalized between November 1, 1975, and February 14, 1976, went to Fort Meade, Maryland. Serum specimens not depleted by routine studies were stored. Stored serum specimens from 74 Fort Dix trainees were identified at Fort Meade and forwarded to WRAIR; 39 (53%) of the trainees had been hospitalized after January 1, 1976. These serum samples were initially tested against A/Mayo Clinic antigen. Serum samples with ≥4-fold rises in titer were re-tested against A/New Jersey and A/Victoria/3/75 (H3N2) antigens (*7*). HAI titers to A/Mayo Clinic and A/New Jersey differed only slightly.

Concerns that influenza A (H3N2) infection or vaccination might stimulate antibody to A/Mayo Clinic were addressed. Four groups were studied to identify persons with ≥4-fold heterotypic HAI antibody increases to A/Mayo Clinic. None were found in 39 Fort Dix soldiers who received influenza vaccine in February 1976 (group 1), and none were found among 27 hospitalized soldiers from posts other than Fort Dix who had ≥4-fold rises in complement fixation (CF) antibody to influenza A (group 2) (*7*). In the third group, ≥4-fold rises in antibody titers developed in 3 (8%) of 40 soldiers from Fort Dix and elsewhere who had been hospitalized with an A/Victoria isolate (*7*). In the fourth group, a single serum sample was studied from each of 168 randomly selected Fort Dix basic trainees who had received their annual influenza vaccination 3 to 4 weeks earlier (*11*). Only 4 (2%) had HAI titers ≥1:20 to A/Mayo Clinic (*11*). In similar studies by others, in 0%–6% of persons, heterotypic antibody to influenza A/swine developed after infection with A/Victoria (H3N2) or influenza vaccination (*12*,*13*).

Since heterotypic antibody to A/Mayo Clinic seldom occurred, soldiers who were hospitalized for acute respiratory disease and showed a ≥4-fold titer rise to influenza A (Hsw1N1) in stored serum specimens from the Adenovirus Surveillance Program were considered to have had A/New Jersey infections. Eight new cases in basic trainees were found. Three (38%) of the 8 solders also had ≥4-fold antibody rises to A/Victoria. Therefore, 13 male, enlisted soldiers, aged 17–21 years, were identified as having had respiratory diseases resulting in hospitalization or death and an A/New Jersey (Hsw1N1) isolate or serologic conversion to A/New Jersey (case-patients). Ten had arrived at Fort Dix between January 5 and February 3, 1976. Three arrived between September 9 and December 30, 1975. Dates of onset of illness were known for 12 and were from January 12 to February 8, 1976. Hospital admissions occurred between January 19 and February 9. Autopsy findings for the only patient who died showed severe edema, hemorrhage, and mononuclear infiltrates in the

lungs, consistent with viral pneumonia. No preexisting disease or bacterial infection was found. Four (33%) of the 12 surviving patients had radiologic evidence of pneumonia but their clinical syndromes were similar to those described for patients with infections caused by other influenza A strains (*7*).

Twelve of the 13 patients were basic trainees; one was an office worker who had an A/New Jersey isolate (*7*). The 12 trainees were in 9 different training companies (*7*,*14*). One company had 3 patients, and 1 company had 2 patients. In these 2 companies, all patients came from the same platoon. Nine were interviewed. Except for those in the same unit, the patients were unknown to each other. All denied swine contact for 6 months before admission. No common variables in working or living environments were identified. All had contact with the Fort Dix medical care system, but care took place in 5 clinics and 2 wards. From January 19 to February 9, there were 7 days when none occupied a hospital bed (*7*,*14*).

Transmission and Illness in Units with Case-patients

Transmission was assessed by using HAI antibody titers to A/Mayo Clinic (Hsw1N1). Sixteen of 17 contacts of the patient not in basic training, 18–43 years of age, were studied, and 4 (25%) had titers ≥1:20 (*14*). One of the 9 training companies had a case-patient who completed basic training before the case was identified and was not studied. In another company with a case-patient, 13 soldiers were studied, and all had titers ≤1:10, but their platoons were not identified. Seven companies were studied by comparing the platoon with at least 1 case-patient to other platoons in the company. Some members of all 7 platoons with case-patients had titers ≥1:20, varying from 7% to 56% (median = 26%). In other platoons from these seven companies, the prevalence of titers ≥1:20 ranged from 0% to 40% (median 18%), which indicated that A/New Jersey virus transmission was not limited to 1 platoon in most companies (*14*).

Comparable samples of soldiers from the 7 companies with cases discussed above and 7 contemporary companies without cases were evaluated. Prevalences of HAI antibody titers to A/Mayo Clinic ≥1:20 in the companies with cases ranged from 0% to 45% (median 18%) (*8*). Prevalences in the companies without cases was 0%–10% (median 4%) (*14*).

Available records permitted the identification of hospital admissions for acute respiratory disease in 6 of the 9 companies with an A/New Jersey case. From January 19 to February 9, 1976, when the A/New Jersey patients from these companies were admitted, admission rates for acute respiratory disease of ≥3.0 per 100 men per week were observed in 4 of the companies. The highest rates occurred during the week ending January 25 and ranged from 1.1 to 6.9 (median 3.4) per 100 men per week (*14*).

Extent of Spread and Duration of Outbreak

The weekly formation of segregated cohorts of new recruits provided an opportunity to study the extent and duration of virus transmission. A random 9% sample of soldiers beginning basic training from January 5 to March 1 were studied for HAI antibody to A/Mayo Clinic (Hsw1N1) (*11*). The prevalence of titers ≥1:20 by weekly cohort ranged from 0% to 19%. The 3 highest prevalences, 19%, 12%, and 9%, occurred in cohorts who started training on January 12, 19, and 26, respectively. Prevalences for 6 other cohorts ranged from 0% to 5%, with 0% prevalence in the cohorts that started training on January 5 and March 1 (*11*). Eleven of the 12 Fort Dix basic training soldiers identified as A/New Jersey case-patients also began training on January 12, 19, and 26 (*11*,*14*).

From February 21 to February 27, a total of 39 soldiers in the basic training reception center were studied for HAI antibody to A/New Jersey (Table) (*11*). This same group was studied 5 weeks later. All 39 had HAI antibody titers to A/Mayo Clinic <1:10 initially and at 5 weeks. The prevalence of HAI antibody titers to A/Mayo Clinic antigen was also determined in advanced training students, civilians who visited the Fort Dix Phlebotomy Clinic, installation maintenance workers, basic training instructors, military medical and veterinary personnel, and soldiers who worked in the reception center. In advanced training students and persons ≤25 years of age, the prevalence of titers ≥1:20 was 0%–6%, consistent with heterotypic responses. However, titers were higher in persons ≥26 years old; most had prevalences in the range of 17% to 44%, but women and men ≥51 years of age at the Phlebotomy Clinic had prevalences of 92% (n = 37) and 88% (n = 60), respectively (*11*).

The earliest A/New Jersey patient was hospitalized on January 19; the last identified patient was admitted on February 9 (Table) (*7*). Both were identified by serologic testing. Four of 5 patients with virus isolates were admitted on January 29 and 30. The last A/New Jersey isolate came from the soldier who died on February 4. The patient admitted on January 19 reported that his onset of illness occurred on January 12. Since no evidence was found for A/New Jersey virus at Fort Dix before January 12, the virus was likely introduced on or shortly after resumption of training on January 5. As shown by the clustering of hospital admissions, the A/New Jersey outbreak peaked during late January and tapered off in early February. The absence of any indication of the A/New Jersey virus in the cohort beginning basic training on March 1 and in the reception center group who gave blood samples from February 21 to February 27 and 5 weeks later supports the

conclusion that A/New Jersey disappeared in February (Table) (*11*).

To understand the relationship of the A/Victoria and A/New Jersey/76 (Hsw1N1) outbreaks, serum specimens from the 9% sample of soldiers who began basic training from January 5 to March 1 were also studied for HAI antibody to A/Victoria. The geometric mean titers to A/Victoria ≥1:10 for cohorts beginning training on January 5 and January 12 were 1:56 and 1:53, respectively. The geometric mean titers then increased to 1:114 in the cohort that started on February 2, peaked at 1:120 in the cohort that began on February 9, remained high at 1:109 for the February 16 cohort, and then returned to baseline (*11*). Thus, the A/New Jersey outbreak likely started in early January and peaked in late January, followed closely by the A/Victoria outbreak.

Even though A/Mayo Clinic titers ≥1:20 were seen in Fort Dix populations other than basic trainees, the prevalences in young people were very low, consistent with heterotypic antibody. Higher prevalences in older persons could have been related to earlier influenza A (H1N1) infections or vaccinations with vaccines that contained swine influenza antigens (*10*). The high titers to A/Mayo Clinic in these groups could not be related to illness, vaccination, or swine contact (*11*). When the serologic data were extrapolated, the total number of A/New Jersey infections in Fort Dix basic trainees was ≈230 when contacts of all 13 case-patients were considered and ≈142 when only virologically confirmed cases were considered true cases (*11*,*15*).

Case Finding beyond Fort Dix

Influenza A/Victoria-like strains had been identified in New Jersey as early as January 21, 1976. By the end of January, the state had investigated reports of high employee and student absenteeism and a hospital outbreak. Patients in all episodes were sampled by using virus isolation and serologic testing. All laboratory reports indicated A/Victoria virus infections (*4*).

Starting February 10, arrangements were made to study febrile respiratory disease patients at McGuire Air Force Base (adjoining Fort Dix) and at hospitals, emergency rooms, and physicians' offices in the Fort Dix vicinity. Medical examiners were told to obtain specimens from possible influenza patients and surveillance was increased statewide. From January 9 to March 19, infection with influenza A/Victoria virus was documented in 301 persons by virus isolation (151 persons), CF or HAI serology (113 persons), or both (37 persons). Cases in New Jersey came from 19 of 21 counties, McGuire Air Force Base, and Lakehurst Naval Training Center. Delaware had 19 cases, including 5 from Dover Air Force Base. From January 31 to March 17, 10 civilian deaths in New Jersey were attributed to influenza. Influenza A/Victoria (H3N2) was isolated from all 10 patients (*4*).

The numbers of isolation and serologic specimens tested and the percentages positive for A/Victoria were consistent with an outbreak that began quickly in January and declined in late February to early March. No influenza cases were identified after March 19; influenza A/New Jersey was never isolated outside Fort Dix (Table) (*4*,*7*).

Among patients with serologic evidence of influenza, HAI antibody responses to both A/Victoria and A/New Jersey were studied in 134. Six (4%), aged 22 to 71 years, had ≥4-fold HAI rises in titer to both viruses (*4*). In the absence of any association with swine influenza A virus, the A/New Jersey titers were attributed to A/Victoria infections.

Summary and Speculation

A/New Jersey/76 (Hsw1N1) was likely introduced into Fort Dix early in 1976, after the holidays (*15*). The virus caused disease with radiologic evidence of pneumonia in at least 4 soldiers and 1 death; all of these patients had previously been healthy (*7*,*15*). The virus was transmitted to close contacts in the unique basic training environment, with limited transmission outside the basic training group. A/New Jersey probably circulated for a month and disappeared. The source of the virus, the exact time of its introduction into Fort Dix, and factors limiting its spread and duration are unknown (*15*).

The Fort Dix outbreak may have been a zoonotic anomaly caused by introduction of an animal virus into a stressed population in close contact in crowded facilities during a cold winter. However, the impact of A/New Jersey virus on this healthy young population was severe in terms of estimated infections, hospitalizations, and duration of the outbreak.

If the outbreak was more than an anomaly, why did it not extend beyond basic trainees? Several factors merit consideration. Contact between basic trainees and others was limited. Moreover, a swine influenza antigen was included in annual military influenza vaccine formulations from 1955 through 1969 (*10*). The high antibody titers to A/Mayo Clinic antigen observed with increasing age in the Phlebotomy Clinic population may reflect earlier influenza A (H1N1) infections or vaccine exposure and some protection (*11*). Also, competition between A/New Jersey and A/Victoria viruses must be considered. The A/Victoria virus spread widely and may have limited the impact of A/New Jersey virus with its lesser ability for human transmission.

Could the Fort Dix outbreak have resulted from interaction between swine influenza A and A/Victoria viruses? A/Victoria transmission occurred in New Jersey before A/New Jersey was identified at Fort Dix. Is it possible that

A/Victoria virus and an early A/New Jersey virus coinfected a soldier with genetic exchange, resulting in a recombinant virus with enhanced human transmission capability? The rapid disappearance of A/New Jersey prohibited studies of virus interactions. Genetic analyses of A/New Jersey, A/Victoria and contemporary A/swine viruses might elucidate a relationship.

Communication and collaboration existed at the onset of the outbreak and continued throughout the investigation. The points of contact at the NJ Department of Health, Fort Dix, CDC, and WRAIR had been established before the outbreak, so time was not lost identifying organizations and persons who needed to be contacted. Organizational roles were defined early and respected. The development of outbreak investigation plans, collaboration in field and laboratory work, and exchange of information occurred smoothly. An important part of the Army investigation was establishment of points of contact at WRAIR who communicated with military leaders, the NJ Department of Health, CDC, and the press. Military epidemiology and laboratory teams reported to WRAIR points of contact. This system protected these teams from disruptive inquiries.

The burden on the laboratories supporting this investigation was intense, lasting for weeks. In 1976, WRAIR was a research and field epidemiology laboratory that also operated as a public health reference laboratory. The WRAIR commander had the authority to reallocate and mobilize scientists and laboratory resources. Today, WRAIR no longer functions as a public health laboratory. The depth of resources and flexibility that existed at WRAIR in 1976 cannot be found in other military laboratories (*16*). Duplicating the 1976 laboratory effort today, in timely fashion, would be difficult.

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Dr Gaydos is director, Public Health Practices, for the US Department of Defense Global Emerging Infections Surveillance and Response System. His research interests include acute respiratory diseases in previously healthy persons and sexually transmitted infections.

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Address for correspondence: Joel C. Gaydos, US Department of Defense Global Emerging Infections Surveillance and Response System, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500, USA;. fax: 301-319-9213; email: Joel.Gaydos@na.amedd.army.mil.

Reflections on the 1976 Swine Flu Vaccination Program

David J. Sencer* and J. Donald Millar†

In 1976, 2 recruits at Fort Dix, New Jersey, had an influenzalike illness. Isolates of virus taken from them included A/New Jersey/76 (Hsw1n1), a strain similar to the virus believed at the time to be the cause of the 1918 pandemic, commonly known as swine flu. Serologic studies at Fort Dix suggested that >200 soldiers had been infected and that person-to-person transmission had occurred. We review the process by which these events led to the public health decision to mass-vaccinate the American public against the virus and the subsequent events that led to the program's cancellation. Observations of policy and implementation success and failures are presented that could help guide decisions regarding avian influenza.

"Flu to the Starboard! Man the Harpoons!
Fill with Vaccine! Get the Captain! Hurry!"
Edwin D. Kilbourne, New York Times, February 13, 1976 (*1*)

"Grounding a Pandemic"
Barack Obama and Richard Lugar,
New York Times, June 6, 2005 (*2*)

"It has been 37 years since the last influenza pandemic, or widespread global epidemic, so by historic patterns we may be due for another."
New York Times, July 17, 2005 (*3*)

Kilbourne in 1976 (*1*) noted that pandemics of influenza occur every 11 years. Since the latest prediction in the New York Times (*3*) suggests that after 39 years we may be overdue for a pandemic, and since 2 US senators have recently headlined the possibility (2), that observation may become a political fact. Whether it becomes a scientific fact and a policy fact is yet to be seen. Some reflections on 1976 from 2 insiders' viewpoints may identify some of the pitfalls that public health policymakers will face in addressing potential influenza pandemics.

*Atlanta, Georgia, USA; and †Murraysville, Georgia, USA

Swine Flu at Fort Dix

On February 3, 1976, the New Jersey State Health Department sent the Center for Disease Control (CDC) in Atlanta isolates of virus from recruits at Fort Dix, New Jersey, who had influenzalike illnesses. Most of the isolates were identified as A/Victoria/75 (H3N2), the contemporary epidemic strain. Two of the isolates, however, were not typeable in that laboratory. On February 10, additional isolates were sent and identified in CDC laboratories as A/New Jersey/76 (Hsw1N1), similar to the virus of the 1918 pandemic and better known as "swine flu."

A meeting of representatives of the military, the National Institute of Health, the Food and Drug Administration (FDA), and the State of New Jersey Department of Health was quickly convened on Saturday, February 14, 1976. Plans of action included heightened surveillance in and around Fort Dix, investigation of the ill recruits to determine if contact with pigs had occurred, and serologic testing of recruits to determine if spread had occurred at Fort Dix.

Surveillance activities at Fort Dix gave no indication that recruits had contact with pigs. Surveillance in the surrounding communities found influenza caused by the current strain of influenza, A/Victoria, but no additional cases of swine flu. Serologic testing at Fort Dix indicated that person-to-person transmission had occurred in >200 recruits (*4*).

In 1974 and 1975, 2 instances of humans infected with swine influenza viruses had been documented in the United States. Both persons involved had close contact with pigs, and no evidence for spread of the virus beyond family members with pig contact could be found (*5*).

The National Influenza Immunization Program

On March 10, 1976, the Advisory Committee on Immunization Practices of the United States Public Health Service (ACIP) reviewed the findings. The committee concluded that with a new strain (the H1N1 New Jersey strain)

that could be transmitted from person to person, a pandemic was a possibility. Specifically, the following facts were of concern: 1) persons <50 years of age had no antibodies to this new strain; 2) a current interpandemic strain (A/Victoria) of influenza was widely circulating; 3) this early detection of an outbreak caused by A/New Jersey/76/Hsw1N1 (H1N1) provided an opportunity to produce a vaccine since there was sufficient time between the initial isolates and the advent of an expected influenza season to produce vaccine. In the past when a new pandemic strain had been identified, there had not been enough time to manufacture vaccine on any large scale; 4) influenza vaccines had been used for years with demonstrated safety and efficacy when the currently circulating vaccine strain was incorporated; 5) the military vaccine formulation for years had included H1N1, an indication that production was possible, and no documented adverse effects had been described.

ACIP recommended that an immunization program be launched to prevent the effects of a possible pandemic. One ACIP member summarized the consensus by stating "If we believe in prevention, we have no alternative but to offer and urge the immunization of the population." One ACIP member expressed the view that the vaccine should be stockpiled, not given.

Making this decision carried an unusual urgency. The pharmaceutical industry had just finished manufacture of the vaccine to be used in the 1976–1977 influenza season. At that time, influenza vaccine was produced in fertilized hen's eggs from special flocks of hens. Roosters used for fertilizing the hens were still available; if they were slaughtered, as was customary, the industry could not resume production for several months.

On March 13, an action memo was presented to the Secretary of the Department of Health Education and Welfare (DHEW). It outlined the problem and presented 4 alternative courses of action. First was "business as usual," with the marketplace prevailing and the assumption that a pandemic might not occur. The second was a recommendation that the federal government embark on a major program to immunize a highly susceptible population. As a reason to adopt this plan of action, the memo stated that "the Administration can tolerate unnecessary health expenditures better than unnecessary death and illness if a pandemic should occur." The third proposed course of action was a minimal response, in which the federal government would contract for sufficient vaccine to provide for traditional federal beneficiaries—military personnel, Native Americans, and Medicare-eligible persons. The fourth alternative was a program that would represent an exclusively federal response without involvement of the states.

The proposal recommended by the director of CDC was the second course, namely, for the federal government to contract with private pharmaceutical companies to produce sufficient vaccine to permit the entire population to be immunized against H1N1. The federal government would make grants to state health departments to organize and conduct immunization programs. The federal government would provide vaccine to state health departments and private medical practices. Since influenza caused by A/Victoria was active worldwide, industry was asked to incorporate the swine flu into an A/Victoria product to be used for populations at high risk.

Before the discussions with the secretary of DHEW had been completed, a member of his staff sent a memo to a health policy advisor in the White House, raising the specter of the 1918 pandemic, which had been specifically underemphasized in the CDC presentation. CDC's presentation highlighted the pandemic potential, comparing it with the 1968–69 Hong Kong and 1957–58 Asian pandemics. President Gerald Ford's staff recommended that the president convene a large group of well-known and respected scientists (Albert Sabin and Jonas Salk had to be included) and public representatives to hear the government's proposal and make recommendations to the president about it. After the meeting, the president had a press conference, highlighted by the unique simultaneous appearance of Salk and Sabin. President Ford announced that he accepted the recommendations that CDC had originally made to the secretary of DHEW. The National Influenza Immunization Program (NIIP) was initiated.

The proposal was presented to 4 committees of the Congress, House and Senate authorization committees and House and Senate appropriation committees. All 4 committees reported out favorable legislation, and an appropriation bill was passed and signed.

The estimated budgeted cost of the program was $137 million. When Congress passed the appropriation, newspapers mischaracterized the cost as "$1.9 billion" because the $137 million was included as part of a $1.9 billion supplemental appropriation for the Department of Labor. In the minds of the public, this misconception prevailed.

Immediately after the congressional hearing, a meeting of all directors of state health departments and medical societies was held at CDC. The program was presented by CDC, and attendees were asked for comments. A representative from the New Jersey state health department opposed the plan; the Wisconsin state medical society opposed any federal involvement. Otherwise, state and local health departments approved the plan.

Within CDC, a unit charged with implementing the program, which reported to the director, was established. This unit, NIIP, had complete authority to draw upon any resources at CDC needed. NIIP was responsible for relations with state and local health departments (including administration of the grant program for state operations,

technical advice to the procurement staff for vaccine, and warehousing and distribution of the vaccine to state health departments) and established a proactive system of surveillance for possible adverse effects of the influenza vaccines, the NIIP Surveillance Assessment Center (NIIP-SAC). (This innovative surveillance system would prove to be NIIP's Trojan horse.) In spite of the obstacles discussed below, NIIP administered a program that immunized 45 million in 10 weeks, which resulted in doubling the level of immunization for persons deemed to be at high risk, rapidly identifying adverse effects, and developing and administering an informed consent form for use in a community-based program.

Obstacles to the Vaccination Plan

The principal obstacle was the lack of vaccines. As test batches were prepared, the largest ever field trials of influenza vaccines ensued. The vaccines appeared efficacious and safe (although in the initial trials, children did not respond immunologically to a single dose of vaccine, and a second trial with a revised schedule was needed) (*6*). Hopes were heightened for a late summer/early fall kickoff of mass immunization operations.

In January 1976, before the New Jersey outbreak, CDC had proposed legislation that would have compensated persons damaged as a result of immunization when it was licensed by FDA and administered in the manner recommended by ACIP. The rationale given was that immunization protects the community as well as the individual (a societal benefit) and that when a person participating in that societal benefit is damaged, society had a responsibility to that person. The proposal was sent back from a staff member in the Surgeon General's office with a handwritten note, "This is not a problem."

Soon, however, NIIP received the first of 2 crippling blows to hopes to immunize "every man, woman, and child." The first was later in 1976, when instead of boxes of bottled vaccine, the vaccine manufacturers delivered an ultimatum—that the federal government indemnify them against claims of adverse reactions as a requirement for release of the vaccines. The government quickly capitulated to industry's demand for indemnification. While the manufacturers' ultimatum reflected the trend of increased litigiousness in American society, its unintended, unmistakable subliminal message blared "There's something wrong with this vaccine." This public misperception, warranted or not, ensured that every coincidental health event that occurred in the wake of the swine flu shot would be scrutinized and attributed to the vaccine.

On August 2, 1976, deaths apparently due to an influenzalike illness were reported from Pennsylvania in older men who had attended the convention of the American Legion in Philadelphia. A combined team of CDC and state and local health workers immediately investigated. By the next day, epidemiologic evidence indicated that the disease was not influenza (no secondary cases occurred in the households of the patients). By August 4, laboratory evidence conclusively ruled out influenza. However, this series of events was interpreted by the media and others as an attempt by the government to "stimulate" NIIP.

Shortly after the national campaign began, 3 elderly persons died after receiving the vaccine in the same clinic. Although investigations found no evidence that the vaccine and deaths were causally related, press frenzy was so intense it drew a televised rebuke from Walter Cronkite for sensationalizing coincidental happenings.

Guillain-Barré Syndrome

What NIIP did not and could not survive, however, was the second blow, finding cases of Guillain-Barré syndrome (GBS) among persons receiving swine flu immunizations. As of 1976, >50 "antecedent events" had been identified in temporal relationship to GBS, events that were considered as possible factors in its cause. The list included viral infections, injections, and "being struck by lightning." Whether or not any of the antecedents had a causal relationship to GBS was, and remains, unclear. When cases of GBS were identified among recipients of the swine flu vaccines, they were, of course, well covered by the press. Because GBS cases are always present in the population, the necessary public health questions concerning the cases among vaccine recipients were "Is the number of cases of GBS among vaccine recipients higher than would be expected? And if so, are the increased cases the result of increased surveillance or a true increase?" Leading epidemiologists debated these points, but the consensus, based on the intensified surveillance for GBS (and other conditions) in recipients of the vaccines, was that the number of cases of GBS appeared to be an excess.

Had H1N1 influenza been transmitted at that time, the small apparent risk of GBS from immunization would have been eclipsed by the obvious immediate benefit of vaccine-induced protection against swine flu. However, in December 1976, with >40 million persons immunized and no evidence of H1N1 transmission, federal health officials decided that the possibility of an association of GBS with the vaccine, however small, necessitated stopping immunization, at least until the issue could be explored. A moratorium on the use of the influenza vaccines was announced on December 16; it effectively ended NIIP of 1976. Four days later the New York Times published an op-ed article that began by asserting, "Misunderstandings and misconceptions... have marked Government ... during the last eight years," attributing NIIP and its consequences to "political expediency" and "the self interest of government health bureaucracy" (*7*). These simple and sinister

innuendos had traction, as did 2 epithets used in the article to describe the program, “debacle” in the text and “Swine Flu Fiasco” in the title.

On February 7, the new secretary of DHEW, Joseph A. Califano, announced the resumption of immunization of high-risk populations with monovalent A/Victoria vaccine that had been prepared as part of the federal contracts, and he dismissed the director of CDC.

Lessons Learned

NIIP may offer lessons for today’s policymakers, who are faced with a potential pandemic of avian influenza and struggling with decisions about preventing it (Figure). Two of these lessons bear further scrutiny here.

Media and Presidential Attention

While all decisions related to NIIP had been reached in public sessions (publishing of the initial virus findings in CDC’s weekly newsletter, the Morbidity and Mortality Weekly Report (MMWR); New York Times reporter Harold Schmeck’s coverage of the ACIP sessions, the president’s press conference, and 4 congressional hearings), effective communication from scientifically qualified persons was lacking, and the perception prevailed that the program was motivated by politics rather than science. In retrospect (and to some observers at the time), the president’s highly visible convened meeting and subsequent press conference, which included pictures of his being immunized, were mistakes. These instances seemed to underline the suspicion that the program was politically motivated, rather than a public health response to a possible catastrophe.

Annex 11 of the draft DHEW pandemic preparedness plan states, “For policy decisions and in communication, making clear what is not known is as important as stating what is known. When assumptions are made, the basis for the assumptions and the uncertainties surrounding them should be communicated” (*11*). This goal is much better accomplished if the explanations are communicated by those closest to the problem, who can give authoritative scientific information. Scientific information coming from a nonscientific political figure is likely to encourage skepticism, not enthusiasm.

Neither CDC nor the health agencies of the federal government had been in the habit of holding regular press conferences. CDC considered that its appropriate main line of communication was to states and local health departments, believing that they were best placed to communicate with the public. MMWR served both a professional and public audience and accounted for much of CDC’s press coverage. In 1976, no all-news stations existed, only the nightly news. The decision to stop the NIIP on December 16, 1976, was announced by a press release from the office of the assistant secretary for health. The decision to reinstitute the immunization of those at high risk was announced by a press release from the office of the secretary, DHEW.

1. Expect the unexpected: it will always happen.

Some examples:
- Children did not respond to the initial formulation of vaccine.
- Liability for untoward events after immunization became a major issue.
- Deaths occurred in Pittsburgh that were coincidental with but unrelated to the vaccines (*8*).
- Cases of a new and unrelated disease, Legionnaires disease, appeared (*9*).
- "Excess" cases of Guillain-Barré syndrome appeared among recipients of vaccines (*10*).
- Erroneous laboratory reports of viral isolates or serologic conversions occurred in Washington, DC, Boston, Virginia, and Taiwan.
- The pandemic failed to appear.

2. Surveillance for influenza disease worked well. This was plain, "old-fashioned" surveillance without computers. A new strain of influenza was identified within weeks of the first recognized outbreak of illness.

3. Interagency cooperation works without formal agreements. The state health departments, military, National Institutes of Health, US Food and Drug Administration, and Center for Disease Control all worked together in a cooperative and mutually beneficial manner.

4. Surveillance for untoward events demonstrated that only when large numbers of people are exposed to a vaccine or drug are adverse reactions identified (Guillain-Barré syndrome with influenza vaccines; paralysis with the Cutter poliovirus vaccine in 1955).

5. Health legislation can and should be developed on the basis of the epidemiologic picture.

6. Media and public awareness can be a major obstacle to implementing a large, innovative program responding to risks that are difficult, if not impossible, to quantitate.
- Creating a program as a presidential initiative makes modifying or stopping the program more difficult.
- Explanations should be communicated by those who can give authoritative scientific information.
- Periodic press briefings work better than responding to press queries.

7. The advisability of the decision to begin immunization on the strength of the Fort Dix episode is worthy of serious question and debate (see text).

8. The risk of potentially unnecessary costs in a mass vaccination campaign is minimal. (The direct cost of the 1976 program was $137 million. In today's dollars, this is <$500 million.) The potential cost of a pandemic is inestimable but astronomical.

Figure. Lessons learned from the 1976 National Influenza Immunization Program (NIIP).

In retrospect, periodic press briefings would have served better than responding to press queries. The public must understand that decisions are based on public health, not politics. To this end, health communication should be by health personnel through a regular schedule of media briefings.

Decision To Begin Immunization

This decision is worthy of serious question and debate. As Walter Dowdle (*12*) points out in this issue of Emerging Infectious Diseases, the prevailing wisdom was that a pandemic could be expected at any time. Public health officials were concerned that if immunization was delayed until H1N1 was documented to have spread to other groups, the disease would spread faster than any ability to mobilize preventive vaccination efforts. Three cases of swine influenza had recently occurred in persons who had contact with pigs. In 1918, after the initial outbreak of influenza at Fort Riley in April, widespread outbreaks of influenza did not occur until late summer (*13*).

The Delphi exercise of Schoenbaum in early fall of 1976 (*13*) was the most serious scientific undertaking to poll scientists to decide whether or not to continue the program. Its main finding was that the cost benefit would be best if immunization were limited to those >25 years of age (and now young children are believed to be a potent source of spread of influenza virus!). Unfortunately, no biblical Joseph was there to rise from prison and interpret the future.

As Dowdle further states (*12*), risk assessment and risk management are separate functions. But they must come together with policymakers, who must understand both. These discussions should not take place in large groups in the president's cabinet room but in an environment that can establish an educated understanding of the situation. Once the policy decisions are made, implementation should be left to a single designated agency. Advisory groups should be small but representative. CDC had the lead responsibility for operation of the program. Implementation by committee does not work. Within CDC, a unit was established for program execution, including surveillance, outbreak investigation, vaccine procurement and distribution, assignment of personnel to states, and awarding and monitoring grants to the states. Communications up the chain of command to the policymakers and laterally to other directly involved federal agencies were the responsibility of the CDC director, not the director of NIIP, who was responsible for communications to the states and local health departments, those ultimately implementing operations of the program. This organizational mode functioned well, a tribute to the lack of interagency jealousies.

Decision-making Risks

When lives are at stake, it is better to err on the side of overreaction than underreaction. Because of the unpredictability of influenza, responsible public health leaders must be willing to take risks on behalf of the public. This requires personal courage and a reasonable level of understanding by the politicians to whom these public health leaders are accountable. All policy decisions entail risks and benefits: risks or benefits to the decision maker; risks or benefits to those affected by the decision. In 1976, the federal government wisely opted to put protection of the public first.

Dr Sencer was director of CDC from 1966 to 1977.

Dr Millar was director of NIIP in 1976.

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Address for correspondence: David J. Sencer, 1097 Mason Woods Dr, Atlanta, GA 30329, USA; email: djud@mindspring.com

Influenza Pandemic Periodicity, Virus Recycling, and the Art of Risk Assessment

Walter R. Dowdle*

Influenza pandemic risk assessment is an uncertain art. The theory that influenza A virus pandemics occur every 10 to 11 years and seroarcheologic evidence of virus recycling set the stage in early 1976 for risk assessment and risk management of the Fort Dix, New Jersey, swine influenza outbreak. Additional data and passage of time proved the theory untenable. Much has been learned about influenza A virus and its natural history since 1976, but the exact conditions that lead to the emergence of a pandemic strain are still unknown. Current avian influenza events parallel those of swine influenza in 1976 but on a larger and more complex scale. Pre- and postpandemic risk assessment and risk management are continuous but separate public health functions.

"I am sure that what any of us do, we will be criticized either for doing too much or for doing too little…. If an epidemic does not occur, we will be glad. If it does, then I hope we can say… that we have done everything and made every preparation possible to do the best job within the limits of available scientific knowledge and administrative procedure."

—US Surgeon General Leroy Burney, Meeting of the Association of State and Territorial Health Officers, August 28, 1957 (*1*)

In 1941, on the eve of US entry into World War II, concern about a repeat of the 1918 influenza pandemic and its effect on armed forces led the US military to establish the Commission on Influenza (later combined with other commissions to become the present Armed Forces Epidemiological Board) and place high priority on developing a vaccine (*2*). Pandemic influenza did not materialize, but the vaccine did. The first successful large-scale influenza vaccine field trials were completed in 1943 (*3*). In 1947, failure of the vaccine to provide protection against the epidemic influenza type A antigenic variant confirmed concerns of vaccine obsolescence and led to the term "antigenic shift" (*4*) and designation of the 1947 FM1 strain by the Commission on Influenza as subgroup A′ on the basis of the hemagglutination inhibition (HI) test.

In May 1957, with reports of a potential influenza pandemic in the Far East, risk assessment responsibilities of the Commission on Influenza were clear. The Department of Defense influenza immunization policy of 1954 mandated quick formulation and provision of a new vaccine. The Public Health Service had no such official policy and found risk assessment to be a challenging process that relied heavily on international sources for surveillance and the Influenza Commission for advice. "There was no indication it would become a killer of the 1918 variety, but neither was there positive assurance it would not" (*1*). Risk management was contingent on evidence of "continued low mortality" or "increased virulence" (*1*). The consensus by late June was probable sporadic local occurrences during the summer with an epidemic during fall or winter that would bring only a relatively small increase in deaths. On August 28, the Surgeon General recommended immunization through established physician-patient channels. The watchword was to "alert but not alarm" the public and to generate interest in receiving the vaccine (*1*).

The 1957 Asian virus pandemic simultaneously increased knowledge of influenza pandemics and the complexity of future pandemic risk assessments. The pandemic had appeared exactly 10 years after appearance of the A′ virus, which suggested pandemic periodicity (*5*). Preexisting HI antibodies to the 1957 A2/Asian virus in sera collected before the pandemic were reported for some persons >75 years of age, which suggested that human influenza viruses were recycling (*6*).

*The Task Force for Child Survival and Development, Decatur, Georgia, USA

In July 1968, with reports of influenza epidemics again appearing in the Far East, the US Military Commission on Influenza quickly obtained strains and recommended a new vaccine (*2*). Risk assessment by the Public Health Service this time around was a much simpler process. Annual vaccine recommendations to physicians for persons at high risk for death or severe complications were by now a matter of course. The need for a new vaccine was apparent (*7*), but early reports consistently described the disease as mild (*8*), and the US epidemic was over before the A2/Hong Kong virus was recognized as an antigenic shift (*9,10*).

The 1968 pandemic added to the complexities of risk assessment. The new subtype had appeared, right on time, 11 years after the 1957 Asian pandemic and replaced the dominant influenza A2/Asian virus subtype, as had the viruses of 1947 and 1957. Further, most persons >85 years of age had preexisting antibodies to the 1968 virus, which suggested that the hemagglutinin of this virus, as well as that of the 1957 virus, had appeared previously in the human population (*11*).

In 1976, speculation was rife that a new pandemic strain was due in a few years. The concept of 10- to 11-year influenza A virus pandemic patterns, with disappearance of the predecessor virus, seemed entrenched in the influenza literature. Previous influenza pandemics had occurred in 1968, before that in 1957, and before that in 1947; carrying the logic further, pandemics also occurred in 1929, 1918, 1900, and 1890 (*12*). The concept was supported by the World Health Organization (WHO) classification scheme, which implied that 4 influenza A subtypes had occurred in humans since 1933. In addition, seroarcheologic findings had been interpreted as evidence that the swine virus had last appeared in 1918, the Hong Kong virus (now designated H3) in 1900, and the Asian (H2) virus in 1890, not exactly 10–11 years apart, but in the same order (*13,14*). To some, the next pandemic virus in the sequence was the swine virus of 1918 (*13*).

On February 13, 1976, the New York Times published a guest editorial to remind the public and policy makers that influenza pandemics had marked the end of every decade—every 11 years—since the 1940s. The editorial urged accelerated pandemic planning and coordinated vaccine research (*15*).

Risk Assessment in 1976

Coincidentally, on February 14, 1976, the day after the Times editorial was published, the Center for Disease Control (CDC) hosted an emergency meeting with the US Army, Food and Drug Administration, National Institutes of Health, and New Jersey State Health Department to assess the isolation of swine influenza virus from the late January outbreak at Fort Dix, New Jersey (*16*). Information was insufficient at the time to assess whether the swine influenza virus outbreak was a unique event in susceptible young recruits or the beginning of a pandemic, but the isolation of a predicted potential pandemic strain almost on schedule did not go unnoticed.

On March 10, the Army provided data to the US Advisory Committee on Immunization Practices that confirmed person-to-person transmission of swine influenza virus (*17*). The single swine influenza death loomed large, although most cases were mild. No one at the advisory committee meeting equated the disease potential of this virus with 1918, but the association of swine influenza virus with the most devastating pandemic in memory was widely speculated in the news media. Slightly more than a month after the outbreak, no evidence suggested that a pandemic would or would not occur; a situation such as the Fort Dix outbreak had never been encountered. On March 18, the action memo from the Assistant Secretary of Health to the Secretary, Department of Health, Education, and Welfare stated that "severe epidemics, or pandemics, of influenza occur at approximately 10-year intervals" and publicly linked swine flu with the pandemic of 1918 (*18*).

When WHO convened a meeting of consultants in Geneva on April 7 (*19*), 3 months had passed without evidence of further swine virus transmission anywhere in the world. The swine A/New Jersey strain had not replaced the current A/Victoria strain, which continued to circulate at Fort Dix well into February, and no evidence of swine/Victoria virus reassortants had been seen. Theories of what might happen were being overtaken by the realities of what was happening. The Fort Dix outbreak was beginning to look like an isolated event.

A report from the United Kingdom on the behavior of swine influenza virus in infected human volunteers would not appear in Nature for some weeks (*20*), but in April early rumors circulated that swine A/New Jersey virus was more infectious than classic swine virus but that the symptoms were mild to moderate. The report added little to risk assessment; the findings were consistent with events seen in the outbreak. But an accompanying editorial in Nature summarized the UK and likely European view, which urged caution in vaccine stockpiling and immunization programs and continuing assessment, "until the shape of things to come can be seen more clearly" (*21*).

Beginning in April and continuing into May, a group of US investigators used the Delphi technique to obtain an expert risk assessment with minimal bias (*22*). The 15 participating scientists and epidemiologists concluded that if swine influenza virus were to circulate in the United States, the epidemic would more likely resemble those of 1957 and 1968 than that of 1918. The probability of further swine influenza virus outbreaks was estimated at 0.10.

On August 1, a series of news reports began to appear on a fatal respiratory illness among American Legionnaires attending a convention in Philadelphia (*18*,*23*). Wide but inappropriate speculation that the cause of these unprecedented deaths might be swine influenza, accompanied by equally unprecedented national publicity, precluded further opportunity for rational risk assessment.

Theory of Predictable Pandemics

Unknowingly, at the same time as the Fort Dix outbreak, the Working Group on Pandemic Influenza met in Rougemont, Switzerland, on January 26–28. Issues addressed included the growing body of evidence linking the origin of antigenic shift to animal reservoirs of influenza viruses (*24*), the questionable validity of predictable patterns of pandemic periodicity, and the appropriate classification of the 1947 strain (*25*).

When the 1947 epidemic occurred, only 13 years had passed since the first influenza virus was isolated. Available scientific knowledge was limited. No precedent existed for defining a pandemic strain or distinguishing antigenic shift (a complete change) from antigenic drift (point mutations resulting in accumulated amino acid changes). The 1957 Asian pandemic virus provided the first evidence of a true antigenic shift. The hemagglutinin and neuraminidase surface antigens were totally different from those of their 1956 predecessors. The 1968 Hong Kong pandemic virus provided evidence that antigenic shift can occur in the hemagglutinin independent of the neuraminidase, which was largely unchanged. The 1947 strain failed to meet the definition of an antigenic shift.

The 1971 revision of the system of nomenclature (*26*) recognized the independence of the 2 surface antigens and linked antigenic shifts with influenza A virus subtypes but further confounded the issue by designating the 1947 strain as a subtype for historical reasons. In the 1980 revision, which combined antigenically closely related subtypes regardless of source of isolation (*27*), the previous hemagglutinin designations of swine (Hsw1) and human H0 and H1 subtypes became H1N1, ending a misclassification of the 1947 strain that had endured for >30 years.

Thus, counting 1890, a total of 4 recognized pandemics have been separated by 28 years (1918), 39 years (1957), and 11 years (1968). Excluding the emergence of the H1N1 virus in 1977, an additional 38 years have elapsed since the last pandemic. No predictable pattern of pandemic periodicity exists.

Pandemic Virus Recycling

In 1935, high levels of antibodies to the newly isolated influenza viruses from humans (*28*) and swine (*29*) were commonly seen among persons >10 years of age, which suggested that the 1918–1920 pandemic had been caused by the same or a closely related virus. The birth dates associated with the peak prevalence of swine virus (H1) antibodies did not change in sera collected 12, 17, or 20 years later (*30*). The seroarcheologic findings were validated in 1999 by sequencing the HA gene recovered from persons who died of influenza during the pandemic (*31*). Thus, swine (H1) virus was present from 1918 to 1920 and left a lifelong immunologic imprint on most persons who were ≤25 years of age at the time. Validation of the H1 seroarcheologic model allowed reexamination of earlier reports of preexisting H2 (1957) and H3 (1968) antibodies in sera collected from elderly persons before the respective pandemics (*32*).

Serologic Findings Linking 1890 with H3

After 1957, preexisting H2 antibodies were not commonly observed. Three laboratories reported preexisting H2 antibodies among the elderly, while 3 other laboratories found no orientation of H2 antibody toward any particular age group. Further, peak antibody prevalence from the 2 primary laboratories (*6*,*30*) differed by nearly 8 years. The lack of agreement among investigators and the low levels and low titers of H2 antibodies suggest either differences in test specificity, sensitivity, or both. More recent application of the seroarcheologic model failed to confirm the proposed link of preexisting H2 antibodies with the 1890 pandemic (*32*).

In contrast, preexisting high levels of H3 antibodies among persons >85 years of age in 1968 were common findings in all serologic tests. Some investigators linked the origin of preexisting H3 antibodies to the minor 1900 pandemic (*11*,*14*), whereas others favored the 1890 pandemic (*33*). Observations from recent application of the validated H1 seroarcheologic model to published data linked preexisting H3 antibodies to the pandemic of 1890 (*32*).

We can reasonably conclude that the virus (H3) with the highest HI antibody titers and highest peak antibody prevalence (>90%) in the elderly resulted not from an epidemic (1900) but a pandemic (1890). The virus (H2) with the lowest HI antibody titers and seroprevalence (15%–29%) in the elderly is an unlikely candidate for the most severe influenza event of the late 19th century.

Epidemiologic Findings Linking 1890 with H3

Population immunity against the shared neuraminidase (N2) antigens between the 1968 H3N2 pandemic strain and its H2N2 predecessor is believed to have contributed to the low number of deaths observed in 1968 and 1969 (*12*). However, more dramatic was the selective sharp decrease in expected excess deaths among persons born before 1893. In the 1970 wave that followed, no excess deaths occurred in persons born before 1885 (*34*).

Influenza infection rates in 1968 and 1969 among persons born before 1890 were two thirds lower than among persons born after 1899 (*35*), further linking H3 with 1890.

Unclear Evidence for H2 Recycling

No single, simple explanation has been proposed for the reported low levels of preexisting H2 antibodies before 1957. Whether these antibodies, if specific, represented cross-reactions stimulated by a related virus or by the H3 virus itself is uncertain. Evidence against specificity (or at least prevalence) of H2 antibodies is the absence of any obvious protective effect among persons ≥75 years of age during the 1957–1958 pandemic, which is in stark contrast to the strong correlation of prepandemic antibodies with protection in 1968 and 1969 (H3) and 1977 (H1) (*36*).

Linking H2 to 1890 and H3 to 1900 may have been a historical accident. The reports of preexisting low levels of H2 antibody in persons ≥75 years of age predated the H3 findings by 10 years. Thus, H2 antibodies were attributed to the 1890 pandemic, the only accepted pandemic around that period. When preexisting high levels of H3 antibody were recognized in essentially the same age cohort in 1968, the 1890 pandemic slot had already been taken.

Lack of Evidence for H1 Recycling

Researchers have long speculated (*3*) that preexisting H1 antibody among the elderly in 1918 accounted for the well-known "W" excess death curve (*37*). Theories of special protection of the population >40 years of age compete with theories of extraordinary vulnerability of young adults. But given the continued increase in the death rate curve (albeit dampened) among those >65 years of age in 1918 (*37*) and the remarkably low death rate among those with preexisting antibodies in 1968 (H3) and 1977 (H1), evidence of H1 recycling in 1918 is not compelling. With the passage of time and the absence of sera collected from persons >40 years of age before the 1918 pandemic, the issue of H1 recycling is difficult to resolve.

H1 reappeared, of course, in 1977, but evidence suggests that the 1977 H1N1 virus reemergence was not a natural event (*38*,*39*). Transmission of the mild H1N1 for >25 years, primarily among those born after 1957, coupled with the previous natural transmission among persons born before 1956, completes the H1N1 immunologic experience of all age groups. If a natural recycling sequence ever existed, present population immunity precludes H1 as a pandemic candidate for years to come.

Solid evidence of recycling exists for a single subtype, H3, which (likely with an equine N8 neuraminidase [*40*]) caused the pandemic of 1890 and reemerged with the N2 neuraminidase in 1968. Thus, in the last 115 years, the influenza A virus hemagglutinin had recycled in humans at least once, after 79 years. Neuraminidase subtypes during this same period of time were N8 (1890), N1 (1918), and N2 (1957 and 1968) (*40*). No evidence of neuraminidase recycling has been seen.

Lessons from 1976

Swine influenza virus was isolated in the United States from humans for the first time in 1974, just 2 years before the Fort Dix outbreak (*41*). Additional swine virus infections of humans were confirmed by serologic evidence and virus isolation in 1975 and 1976, with a least 1 suggested incident of person-to-person spread other than the Fort Dix outbreak. Increased recognition of swine influenza infections may have been a matter of increased surveillance, number of susceptible humans, or swine virus transmissibility. Human experimental studies (*20*) and virologic findings (*42*) suggest the latter.

Influenza virus eradication in swine was recommended by WHO in 1976 (*19*), but such action was not taken because of major biologic challenges and absence of resources. Today, even if pandemic risk were absent, the economic loss from infected poultry and mounting human illness and death are compelling reasons in themselves to place highest priority on avian influenza virus control.

Risk Assessment Limited by Available Knowledge

The major lesson from 1976 was that increased animal-to-human transmission and major outbreaks of a novel influenza virus do not necessarily lead to pandemics, at least in the short term. However, knowledge of the Fort Dix outbreak and evidence that swine influenza virus/H3N2 reassortants could occur in pigs under conditions of natural transmission (*24*) likely would have generated concerns for years about swine influenza transmission to humans had not H1N1 virus reappeared in the human population in 1977.

Since 1976, available knowledge of the influenza A virus and its natural history has expanded greatly. Multiple experimental studies have better defined conditions for virus mutation and the creation of reassortants. Opportunities for human exposure and the current number of incidences of avian virus transmission to human are unprecedented in modern times, but in 2005, as in 1976, the precise conditions that lead to the emergence of a pandemic strain are unknown.

Concern of Virus Recycling

In recent history, influenza virus recycling has occurred twice, once through the natural process (H3 in1968) and once likely through human negligence (H1 in 1977) (*38*,*39*). If human influenza A epidemics are restricted to 3 subtypes, as some have speculated, and if H1 and H3 are presently in circulation, then only H2 remains. The risk of H2 reemerging in humans through an act of nature is

theoretical. The risk of H2 reemerging through an act of human negligence is all too real.

In the published report of the April 7, 1976, WHO meeting of international experts, the final paragraph urged extreme caution in developing live vaccines from A/New Jersey strains (H1N1) because of the possible danger of spread to susceptible human or animal hosts (*19*). That paragraph was written specifically to respond to reports that several investigators outside Western Europe had plans to develop and test such vaccines. One year later, an H1N1 virus, identical to the laboratory strain from1950–1951, swept the world.

In an incident earlier this year, H2N2 virus was accidentally distributed in proficiency testing panels to laboratories in 18 countries. Recognizing the potential danger, CDC and WHO issued a health advisory on April 13, 2005, to destroy all such samples and followed on May 3 with recommendations to increase biosafety levels for H2N2. Laboratory containment of H2N2 strains is crucial. No one born since 1968, including many laboratory staff, is immune. The level of compliance with these biosafety recommendations in all areas of the world is unknown. Focusing on the theoretical risk for natural H2 emergence and ignoring the real risk in our own laboratories would be tragic.

Risk Assessment Separate from Risk Management

Internationally, influenza risk assessment and risk management are separate functions. WHO makes risk assessments in the form of annual recommendations on influenza vaccine composition. Nations may elect to accept WHO findings and recommendations or to have their own risk assessment bodies that incorporate WHO findings. Risk management, on the other hand, is the exclusive responsibility of national governments. Independent expert bodies may make recommendations, but risk management ultimately is a political process, performed and funded by federal and state governments.

Nationally, risk assessment should also be a separate scientific function, free from influence by perceived risk-management resource constraints, organizational capacities, or political aspirations. Pandemic risk management, itself an uncertain art, must independently weigh ongoing risk-assessment findings in the context of actions that best serve national and international interests.

Dr Dowdle is a member of The Task Force for Child Survival and Development, Atlanta, Georgia. His current scientific interests include polio, HIV, and influenza.

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Address for correspondence: Walter R. Dowdle, Task Force for Child Survival and Development, 750 Commerce Dr, Ste 400, Decatur, GA 30030, USA; fax: 404-371-1087; email: wdowdle@taskforce.org

The Swine Flu Episode and the Fog of Epidemics[1]

Richard Krause*

The 1918 influenza pandemic has shaped research and public health for nearly a century. In 1976, the specter of 1918 loomed large when a pandemic threatened the country again. Public health officials initiated a mass vaccination campaign, but the anticipated pandemic failed to occur. An examination of the available data in 1976 and the decision to vaccinate, as well as lessons learned from the HIV/AIDS epidemic in the early 1980s, may help shape an appropriate public health response to future threats from avian influenza or other infectious diseases.

"Maye it please your Honor immediately upon the Queen's arrival here, she fell acquainted with a new disease that is common in this town, called here the *Newe Acquayantance*, which passed also throughe her whole Courte, neither sparing lordes, ladies nor damoysells, not so much as either Frenche or English… There was no appearance of danger, nor manie that die of the disease, excepte some olde folks. I am ashamed to say that I have byne free of it, seeing it seketh acquayantance at all man's handes."

—Written in a letter in 1562 by Sir Thomas Randolph, ambassador from Queen Elizabeth I to the court of Mary, Queen of Scots, Edinburgh, to Cecil in London (*1*).

I read the 1953 lecture on Influenza: the Newe Acquayantance (*1*) by Thomas Francis, Jr, in 1953, and I did not read it again until recently, as I was preparing this article. On reflection, I wish I had reread it during the swine flu episode in 1976. Certainly Francis's lecture, and his conclusions and speculations about the mysteries of 1918 influenza, should temper our strategies for coping with a possible human pandemic arising, like a phoenix, from the current influenza epidemic in Asian chickens.

In light of an influenza outbreak at Fort Dix, New Jersey, in February of 1976, the Public Health Service decided to prepare an influenza vaccine with the Fort Dix strain and immunize a large segment of the US population. Mass immunization was achieved by October of that year, although the predicted pandemic never occurred.

Now, 30 years later, we are faced with the threat of an influenza pandemic that might emerge from a massive outbreak of avian influenza H5N1 in Asian chickens. Many scientists and public health professionals who must now make decisions about the public health response are not virologists or influenza experts, as I was not, and they will need to base their decisions on expert opinion and their own evaluation of the facts. In 1976, I supported the decision to begin mass immunization, and this article examines the data and experiences that contributed to that decision. I hope my reflections will be useful for those who must determine the public health response to the threat of H5N1 in 2005. They have my best wishes.

Early Experiences with Influenza

At the beginning of the 20th century, the fact that many contagious diseases were caused by microbes was well established, but at the time no treatment was available for any of them except syphilis and malaria. Anxiety and alarm were widespread among those who lived through the devastating 1918 influenza pandemic about the potential for a recurrence. In 1918, my parents and my brothers, then children, were living in a small town in southeastern Ohio. When I was a teenager in the 1930s, I recall my mother's reflections on the influenza pandemic. Our home at the time was near a chair factory, and after work many of the employees walked past our house. Occasionally, a worker would spit phlegm or tobacco on the pavement. For such occurrences, my mother always had a kettle of boiling water ready, so she could immediately scald the "damned spot," hoping to kill the unseen germs and protect my brothers from influenza.

I relate this anecdote as a reminder that as recently as the 1930s, when I was a teenager, the 1918 pandemic was a living memory. To this day, that pandemic casts the

*National Institutes of Health, Bethesda, Maryland, USA

[1]Longer version of this article available from the author Richard_Krause@nih.gov

longest shadow, although the AIDS pandemic will likely take its place.

My next experience with influenza was in 1944, when I was in the US Army. The influenza vaccine had just been developed by Francis, Jonas Salk, and others. Their work had been supported by the army under the auspices of the Armed Forces Epidemiology Board (AFEB), for whom the pandemic of 1918 was still fresh: 50,000 soldiers had died of influenza. We GIs were lined up at the dispensary and given the vaccine, one soldier after another, with the same 50-mL syringe.

To this day, I recall the moderately severe local reaction, swelling, considerable tenderness, and pain at the injection site, and many soldiers had systemic reactions. I remember that the vaccine in the syringe was turbid**,** but did not know at the time that it had been grown in eggs. I have wondered since then if the turbidity of the vaccine was due to a residue of chicken feathers! Clearly, purification had a long way to go in 1944.

Swine Flu

From 1970 to 1974, I was a member of the National Institute of Allergy and Infectious Diseases (NIAID) Infectious Disease Advisory Committee. Several times a year, we reviewed various protocols for evaluating vaccines, including influenza, that were conducted in the vaccine evaluation units then supported by NIAID. We were kept abreast of the efforts to match the influenza virus strains incorporated into the vaccines with the anticipated wild strains that would circulate in the coming season.

In the first months of 1976, mere weeks after I had become director of NIAID, influenza broke out at Fort Dix, New Jersey. Several soldiers died, and soon the Center for Disease Control (CDC) and other agencies determined that the cause was a swine flu virus (H1N1), thought to be a direct descendant of the virus that caused the pandemic of 1918. This conclusion was based on antibodies to H1N1 antigens found in survivors of the 1918 pandemic**,** and the belief that the 1918 virus was eventually transmitted to pigs in the Midwest, where it persisted and caused sporadic human cases. Had the virus broken out of the pigsty, so to speak, and caused the outbreak in humans at Fort Dix?

Approximately 200 young men were infected in January and February, as detected by conversion of serial sera from negative to positive for swine flu hemagglutinins. This finding was reported by Frank Top to the AFEB. With the exception of 1 or 2 deaths, the disease was reported to be mild.

Sometime in February 1976 a group of intramural and extramural influenza experts reached a near consensus that the Fort Dix swine flu was likely to be the source of an imminent pandemic of influenza, perhaps similar to the pandemic of 1918, because Fort Dix virus had the antigenic characteristics of what was thought to be the 1918 virus. One notable exception to this consensus thought it possible but unlikely that the Fort Dix outbreak would be the origin of a pandemic. He noted that an influenza epidemic began like a cloudburst in the population in which it first makes its appearance, for example, in a cluster of schoolchildren, as was the case with Asian flu in 1958.

Predictably, meetings of the experts were called, and a general sense of alarm prevailed, as well as a sense that something must be done to prevent an epidemic that might be a replay of 1918. All agreed that we needed to enhance national and worldwide surveillance to determine the extent of a possible major outbreak of this virus, but other courses of action were more hotly debated. Flu vaccines became available in 1944, and the primary question facing us was whether we should quickly prepare a vaccine with the Fort Dix swine flu virus strain and immunize as much of the population as possible.

In January, and for the next 10 months, David Sencer, director of CDC, frequently consulted with Harry Meyer, director of the Bureau of Biologics, and myself. Also involved in the discussions were Theodore Cooper, assistant secretary for the Department of Health, Education, and Welfare; Hope Hopps, Bureau of Biologics; Walter Dowdle, chief of the virology section at CDC; and John Seal, deputy director of NIAID. William Jordan and John LaMontagne later joined the NIAID circle. Maurice Hilleman of Merck frequently joined an informal group for intense discussions on clinical trials that were conducted in the spring of 1976 with the vaccines that had been quickly prepared by the industry.

Throughout the spring and summer, we monitored carefully for swine flu elsewhere in the world, particularly in the Southern Hemisphere, where it was winter. We received only scattered reports of an occasional case of swine flu in farmers in the Midwest, and controversy raged as to what the next steps should be. Should the vaccine be stockpiled? The argument against stockpiling was strong: the vaccine had to be given before the potential epidemic occurred in September and October, and we were racing against time. Initially, Albert Sabin insisted the vaccine should be given to children when school began in September 1976. Yet some experts preferred a "wait and see" approach.

After much consultation and discussion at the highest levels of the US government, the Public Health Service launched a program to immunize 50 million people. Following the largest voluntary mass vaccination campaign since the mass vaccination programs with Salk and Sabin polio vaccines, nearly 25% of the US population, or 45 million persons, were vaccinated by October, 10 short months after the alarm was sounded.

The epidemic, however, did not occur. The Fort Dix outbreak was a false alarm, and the American public and much of the scientific community accused us of overreacting. As someone noted, 1976 was the first time we had been blamed for an epidemic that did not take place.

Donald Burke and his group at the Johns Hopkins School of Public Health have recently calculated the basic reproductive rate (R_0) of the 1976 virus. On the basis of available historical data, they calculate an R_0 of 1.1–1.2. This number suggests that swine flu would not have become a major epidemic. We did not have those calculations at the time, nor were such calculations widely used. At least R_0 was >1 and not <1.

These efforts to prevent an epidemic were, in some ways, like a big "fire drill." We proved it was possible to organize a mass influenza immunization program from start to finish: identify the virus, grow up stocks, prepare and field test the vaccine, provide for indemnity, and immunize a large segment of the population, all within 10 months. We learned a great deal from that drill, and I am sure we can do better the next time. The day will come when we will again retrace this race against time.

The Fog of Epidemics

The uncertainty that surrounds any response to a microbial outbreak, the "fog of epidemics," is analogous to the fog of war, of which historians speak (*2*).

The Fog of War: Uncertainty
Where is the enemy?
What is his strength?
What counterattack?

The Fog of Epidemics: Uncertainty
Where is the microbe?
How many; how virulent; how communicable?
What counterattack?

Perceived Miscalculations
1975 Swine flu outbreak
Response too rapid
1981 HIV/AIDS occurrence
Response too slow[1]

In the case of swine flu, we may have acted too soon. And in the case of AIDS, we may have acted too slowly. Read the book by Neustadt and Fineberg (*3*) for a full account of our perceived folly in regard to swine flu. For an account of the perception that from 1981 to 1984, as director of NIAID, I dithered over the onset of the HIV/AIDS epidemic, read what Shilts says about me in And the Band Played On (*4*).

I relate these personal reminiscences because many who read this article will be on the firing line when future epidemics threaten, and they may either erupt or fizzle out. You will be in a fog, and you will need to exercise the best judgment you can on the basis of available surveillance information and historical context. Roy Anderson and others have been on the firing line in the United Kingdom with bovine spongiform encephalopathy and foot-and-mouth disease. And now any number of national and international organizations and the ministries of health in many countries in Southeast Asia are on the firing line in regard to avian influenza. Should we stockpile drugs? Prepare a vaccine? Cull infected flocks? When difficult choices arise, criticism is almost certain to follow, but as Harry Truman said, "If you can't stand the heat, stay out of the kitchen."

Original Antigenic Sin

Any narrative on the swine flu episode would be incomplete without mentioning the work of Richard Shope on the possible relationship between the putative influenza virus of 1918 and its eventful residence in pigs in Iowa, where it caused an influenzalike syndrome and where it remained as a reservoir (*5*). Whatever the merits of this argument about the cause of swine flu virus infection in adults in the 1930s, of interest here is Francis's suggestion that the swine flu antibody in humans was the result of repeated exposure to human strains, and perhaps not due to prior infection with the 1918 virus. Surely his thoughts about this matter were the genesis of the concepts expressed in On the Doctrine of Original Antigenic Sin, published in 1960 (*6*).

Francis wrote, "The antibody of childhood is largely a response to dominant antigen of the virus causing the first type A influenza infection of the lifetime. The antibody-forming mechanisms are highly conditioned by the first stimulus, so that later infections with strains of the same type successfully enhance the original antibody to maintain it at the highest level at all times in that age group. The imprint established by the original virus infection governs the antibody response thereafter. This we have called the Doctrine of the Original Antigenic Sin."

Francis died in 1969 and did not live to know the full explanations for antigenic shift through reassortment of gene segments from 2 parent viruses or antigenic drift through mutation. He surely would have been in awe, as we all are, of the molecular explanation of influenza virus variation with succeeding epidemics. And yet, even with

[1]Reprinted from Emerging Infections, Richard M. Krause, Introduction, pages 1–22, Copyright 1998, with permission from Elsevier.

the brilliant work of Taubenberger delineating the 1918 virus (*7*), we can still ask Francis's question: Which strain will cause the next pandemic? Francis would have been cautious, but he certainly would have agreed that knowing the genetics of the 1918 virus will guide our strategy to confront future influenza pandemics. And I believe he would be cautious about the pandemic potential of the current avian influenza virus. He would warn us to keep alert to the unexpected, to be prepared for a "newe acquayantance."

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Dr Krause is a senior investigator in the Laboratory of Human Bacterial Pathogenesis at the National Institute of Allergy and Infectious Diseases, National Institutes of Health. The persistent theme underlying his research concerns the substances in bacteria that stimulate the body's immune system and the genetic factors that influence the immune response. Specifically, he has been involved in wide-ranging studies on the microbiology and immunology of streptococcal infections.

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Address for correspondence: Richard Krause, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg 16, Rm 202, 16 Center Dr, Bethesda, MD 20892, USA; fax: 301-496-8496; email: richard_krause@nih.gov

Antiviral Response in Pandemic Influenza Viruses

Adolfo García-Sastre*

The outcome of viral infections depends on a complex set of interactions between the viruses and their hosts. Particularly, viral infection triggers specific signaling programs within the infected cells that results in substantial changes in host gene expression. While some of these changes might be beneficial for viral replication, others represent the induction of a host antiviral response. In this respect, viruses have evolved genes that counteract this initial innate antiviral response. These viral-host interactions shape the subsequent phases of the disease and influence the adaptive immune response. In influenza viruses, the nonstructural protein 1 inhibits the interferon-mediated antiviral response. The regulatory activities of this viral protein play a major role in the pathogenicity of influenza virus and appear partially responsible for the ability of influenza viruses to infect multiple animal species, which likely contributes to the generation of new pandemic viruses in humans.

Coevolution of pathogens with their hosts has resulted in the shaping of the host immune system. A major component of this system is the innate immune response, which includes all the host barriers and responses with broad specificity against pathogens. The innate immune response not only represents the first barrier against infection but also provides the appropriate signals required for the subsequent adaptive cellular and humoral immune responses to develop. The type I interferon (IFNα/β) response constitutes a critical element of the innate immune system that is particularly important in the battle against viral pathogens. Secretion of IFNα/β results in the induction of a cellular antiviral response involving the transcriptional upregulation of >100 genes (*1*).

Despite the host's sophisticated immune system, viruses continue to successfully infect them and cause disease and, in some cases, death. The success of viruses is explained, at least in part, by the acquisition of viral genes during evolution that antagonize the host immune response. Viral-encoded IFNα/β antagonists are of particular interest, since they appear to be present in most animal viruses. We detail how influenza viruses evade the host innate immunity, with particular emphasis on the IFNα/β response, and the implications of this immune evasion in pandemic influenza.

*Mount Sinai School of Medicine, New York, New York, USA

IFNα/β Antiviral Response

Animal cells that sense viral infection respond almost immediately by synthesizing and secreting IFNα/β. The IFNα/β genes include IFNβ and many closely related IFNα genes. Most cells have intracellular sensors of viral products that, when activated, initiate a signaling cascade that results in transcriptional induction of the IFNβ gene. The nature of these sensors has remained unknown until recently, when 2 putative RNA helicases, RIG-I and MDA-5, were identified as sensors for viral dsRNA generated in the cytoplasm during viral infection (*2*–*4*). Binding to dsRNA by these proteins may result in initiation of helicase activity, concomitant with a conformational change that leads to recruiting additional cellular factors, including the recently identified IPS-1/MAVS protein (*5*,*6*). As a result, different cellular kinases, including the IRF3 kinases TBK1 and IKKε, become activated. Activated IRF3, together with NF-κB and AP-1, accumulate in the nucleus, bind to the IFNβ promoter, and stimulate transcription. While cytoplasmic viral dsRNA is one of the viral molecules that trigger this cascade, other viral products and other cellular sensor molecules also likely participate in the induction of IFNβ. IFNα/β induction is also stimulated by the presence of viral RNA and DNA in the endosome through the action of TLR3, TLR7, TLR8, or TLR9 (*7*). Different cell subtypes appear to employ different mechanisms to recognize viral products (*8*).

Once IFNα/β has been synthesized, it is secreted and binds to the IFNα/β receptor. All IFNαs and IFNβ bind to the same receptor and as a result, the cytoplasmic kinases

JAK1 and TYK2 become activated and phosphorylate the STAT1 and STAT2 molecules. This process promotes generation of the ISGF3 transcription factor, a complex of STAT1, STAT2, and IRF9 that accumulates in the nucleus. Nuclear ISGF3 binds to promoters that contain interferon-stimulated response elements and stimulates the transcriptional induction of antiviral genes, including MxA, PKR, OAS, ADAR, PML, p56, and many others (*9*). These IFN-stimulated genes inhibit viral replication by many different mechanisms, including binding to viral nucleocapsids, translation inhibition, RNA degradation, RNA editing, and apoptosis induction. Moreover, secreted IFNα/β promotes the generation of robust cellular and humoral immunity (*10,11*). In general, the IFNα/β response has a complex regulation that involves positive and negative feed-back mechanisms, some of which are still unknown.

Nonstructural Protein 1 of Influenza Virus

Although IFNα/β was first described as a factor with antiviral activity secreted by cells treated with partially heat-inactivated influenza A viruses (*12*), it was also recognized early on that influenza viruses are poor IFNα/β inducers (*13*). This is because influenza viruses, like many other viruses, encode mechanisms to evade and antagonize the IFNα/β response (*14*). In the case of influenza A virus, this IFNα/β antagonistic function is encoded by the nonstructural protein 1 (NS1) gene.

NS1 of influenza A viruses is encoded by the unspliced mRNA derived from the shortest RNA segment of the 8 viral RNA segments. The protein is the most abundant nonstructural viral protein expressed in influenza A virus–infected cells. The development of reverse genetics techniques to manipulate the influenza virus genome made it possible to generate NS1 mutant viruses, including a recombinant influenza A virus lacking the NS1 gene (*15*). The NS1 knockout influenza A virus, delNS1, was replication defective in most cells and hosts, except for those lacking a functional IFNα/β system. Most remarkable, delNS1 virus was highly attenuated in mice but replicated and caused disease in STAT1 knockout mice, which lack one of the key transactivator molecules needed for the IFNα/β response (*15*). These results indicate that NS1 is required to overcome the IFNα/β response during influenza A virus infection.

The basis of the IFNα/β antagonistic properties of the NS1 of influenza A virus relies on its ability to prevent IFNβ synthesis; this explains the poor IFNβ–inducing properties of influenza A viruses (*16,17*). In the absence of NS1, influenza A virus becomes a high IFNα/β–inducing virus, and induction of high levels of IFNα/β results in inhibition of replication of delNS1 virus. NS1, by virtue of its dsRNA binding properties, is likely to sequester viral dsRNA produced during viral infection, which prevents recognition of this dangerous molecule by cellular sensors. This model of action is consistent with the ability of NS1 expression to prevent activation of transcription factors involved in the induction of IFNα/β synthesis, including IRF3 (*16*). Moreover, dsRNA binding is required for optimal inhibition of IFNβ production by NS1 (*18*). Similar results were obtained with the NS1 of influenza B virus (*19,20*). However, interactions of NS1 with cellular proteins also likely contribute to its IFNα/β antagonistic functions (*21*). NS1 of influenza A virus, but not of influenza B virus, inhibits cellular factors involved in mRNA processing (*22,23*); this function might also play a role in inhibiting IFNα/β production by influenza A virus (*24*). Finally, NS1 has also been shown to have IFNα/β inhibitory properties at a post-IFNα/β synthesis level. The NS1 of both influenza A and B viruses prevents the activation of the translation inhibitory and IFN inducible protein PKR (*25,26*); the NS1 of influenza B virus inhibits the activity of ISG15 (*27*), an IFN-inducible protein that enhances the IFN-mediated antiviral response.

Role of NS1 Gene

Influenza A viruses can infect many different animal species, such as different birds (e.g., waterfowl, chickens, turkeys), horses, pigs and humans, but also cross species, with avian strains infecting mammalian species, including humans. This property is especially critical during human pandemics that are characterized by novel antigenic determinants. These determinants derive from avian strains for which no immunity exists in most human population, which results in higher illness and death rates. The factors involved in the ability of an avian influenza virus strain, or of a reassortant virus containing avian antigenic determinants, to infect and propagate in humans are poorly understood; this lack of knowledge hampers our ability to predict the pandemic potential of avian influenza virus strains circulating in birds. Although the receptor specificity of the hemagglutinin protein is a factor that appears to be important for human adaptation of avian strains, other poorly understood factors also participate in this adaptation (*28*). With respect to NS1, viral strains from different animal hosts likely have NS1 genes adapted to antagonize the IFNα/β system of their specific host species. This was the case when the NS1 gene of the human influenza A virus that caused the 1918 H1N1 pandemic was compared with the NS1 gene of the mouse-adapted H1N1 influenza A virus strain WSN. Replacement of the NS1 gene of WSN virus with that of the 1918 virus resulted in an attenuated virus in mice, but this virus more efficiently inhibited the IFNα/β system in human cells (*21,29*). This inhibition might be explained by specific interactions of NS1 with host factors that have different sequences depending on the host, with NS1 of a mouse-adapted strain

interacting better with murine factors than with human factors, and vice versa. If this is a general property of NS1 from different influenza virus strains, an avian strain would require adaptation of its NS1 gene to efficiently antagonize the human IFNα/β system. Alternatively, an avian strain would require acquisition by reassortment of an NS1 gene from a human strain to efficiently infect and propagate in humans.

Because mutations that affect NS1 function also have a profound effect on viral pathogenicity, highly pathogenic influenza virus strains may have an NS1 gene with particularly strong IFNα/β antagonistic properties. Moreover, the ability of NS1 to attenuate the activation of different transcription factors during viral infections has implications beyond the inhibition of IFNα/β synthesis. For instance, expression of many other cytokines and molecules involved in activation of dendritic cell function also appear to be regulated by NS1 (*30*). In this respect, the NS1 of the highly pathogenic avian H5N1 viruses circulating in poultry and waterfowl in Southeast Asia might be responsible for an enhanced proinflammatory cytokine response (especially TNFα) induced by these viruses in human macrophages (*31*,*32*). High levels of proinflammatory cytokines are likely to play an important role in the unusual lethality of these viruses in humans. Fortunately, infection with these viruses appears to be rare and the viruses have not been able to efficiently propagate from human to human.

Other Influenza Antagonists of Host Response

Although delNS1 influenza virus is a high inducer of IFNα/β, partial UV inactivation of this virus results in even higher induction of IFNα/β (*33*). These results suggest the presence of additional viral genes besides NS1 that attenuate IFNα/β production during viral infection and that become inactivated by UV. The viral polymerase, possibly through its endonuclease "cap-snatching" activity, might be responsible for this anti- IFNα/β activity (*33*). Further experimentation will be required to evaluate this hypothesis. In any case, the presence of multiple viral genes that cooperatively antagonize the IFNα/β response is not uncommon among the different virus families.

Influenza A virus encodes a second nonstructural polypeptide in virus-infected cells, the PB1-F2 protein (*34*). This protein is encoded by an alternative open reading frame of the PB1 RNA segment, which also directs the synthesis of the PB1 protein, a critical component of the viral polymerase. The PB1-F2 protein localizes to the mitochondria of the infected cells (*35*) where it interacts with 2 components of the mitochondrial permeability transition pore complex, ANT3 and VDCA1, that are thought to play a major role in apoptosis control (*36*). As a result, expression of PB1-F2 sensitizes cells to apoptosis. This process might constitute an important immune evasion strategy. Thus, a PB1-F2-knockout influenza A virus induced less cell death than the wild-type virus in infected human monocytes, which suggests that expression of PB1-F2 affects immune cell function during viral infection (*34*). Although several influenza A virus strains that lack PB1-F2 occur naturally, PB1-F2 likely contributes to viral pathogenicity and might have an important role in determining the severity of pandemic influenza.

NS1 as Target for Antivirals and Vaccines

Our knowledge of NS1 function might be applied in the near future to select for new antiviral compounds against influenza virus. Predictably, small molecules that interfere with the ability of NS1 to bind dsRNA or prevent IFNα/β production will also enhance the host innate immunity against influenza virus, resulting in faster viral clearance. In addition, recombinant influenza viruses with impaired NS1 function might represent efficient live attenuated vaccines against influenza. These viruses can be grown in IFNα/β-deficient substrates to high titers, but they are attenuated in the host (*37*). Moreover, since the inhibitory effects of NS1 attenuate aspects of both innate and adaptive immunity, NS1 mutant viruses appear to be intrinsically more immunogenic (*38*). Recombinant influenza viruses with modified NS1 genes have been developed and have proven to be attenuated and immunogenic in different animal models. These modified viruses might be used in the future as the basis of live vaccines against epidemic and pandemic influenza (*37–40*).

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Dr García-Sastre is a professor in the department of microbiology at Mount Sinai School of Medicine in New York. His studies provided the first description and molecular analysis of a viral-encoded IFN antagonist among negative-strand RNA viruses.

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Address for correspondence: Adolfo García-Sastre, Mount Sinai School of Medicine, Department of Microbiology, Box 1124, 1 Gustave L. Levy Place, New York, NY 10028, USA; fax: 212-534-1684; email: adolfo.garcia-sastre@mssm.edu

Cell-mediated Protection in Influenza Infection

Paul G. Thomas,* Rachael Keating,* Diane J. Hulse-Post,* and Peter C. Doherty*

Current vaccine strategies against influenza focus on generating robust antibody responses. Because of the high degree of antigenic drift among circulating influenza strains over the course of a year, vaccine strains must be reformulated specifically for each influenza season. The time delay from isolating the pandemic strain to large-scale vaccine production would be detrimental in a pandemic situation. A vaccine approach based on cell-mediated immunity that avoids some of these drawbacks is discussed here. Specifically, cell-mediated responses typically focus on peptides from internal influenza proteins, which are far less susceptible to antigenic variation. We review the literature on the role of CD4+ and CD8+ T cell–mediated immunity in influenza infection and the available data on the role of these responses in protection from highly pathogenic influenza infection. We discuss the advantages of developing a vaccine based on cell-mediated immune responses toward highly pathogenic influenza virus and potential problems arising from immune pressure.

Vaccine approaches against respiratory virus infections such as influenza have relied on inducing antibodies that protect against viral infection by neutralizing virions or blocking the virus's entry into cells. These humoral immune responses target external viral coat proteins that are conserved for a given strain. Antibody-mediated protection is therefore effective against homologous viral strains but inadequate against heterologous strains with serologically distinct coat proteins. This distinction is of consequence since many viruses rapidly mutate their coat proteins; an effective humoral response–based vaccine against a form of the virus may be ineffective against next season's variant. In contrast, T cells, which mediate cellular immune responses, can target internal proteins common to heterologous viral strains. This property gives vaccines that induce protective cellular immune responses the potential to protect against heterologous viral strains.

*St. Jude Children's Research Hospital, Memphis, Tennessee, USA

Antigen-specific ligation of T-cell receptors induces effector mechanisms that either directly or indirectly promote lysis of infected cells. Functionally distinct T-cell subsets are broadly identified according to their differential expression of CD4 and CD8 coreceptors. The CD4+ T helper cells are primarily responsible for helping other immune cells through direct cell-cell interactions or by secreting cytokines after recognizing viral peptides bound to major histocompatibility complex (MHC) class II molecules. The cytotoxic T lymphocytes (CTLs) typically express CD8 and induce apoptosis of cells on which they recognize foreign antigens presented by MHC class I molecules, providing a defense against intracellular pathogens such as viruses. This association of phenotype and function is not absolute, since CD4+ cells may exhibit lytic activity, while CD8+ cells secrete antiviral cytokines, notably interferon-γ (IFN-γ) and tumor necrosis factor. Greater understanding of how each subset contributes to protective immunity and how T-cell memory is maintained and recalled in a secondary infection would contribute to development of effective vaccines that use these basic features of the immune response.

Immune Models of Influenza

Influenza is a contagious, acute respiratory disease caused by infection of the host respiratory tract mucosa by an influenza virus (*1*). The influenza A viruses infect host epithelial cells by attaching to a cellular receptor (sialic acid) by the viral surface protein hemagglutinin (HA). The virus is subsequently released because of the action of another surface glycoprotein, the enzyme neuraminidase (NA), several hours after infection.

Mouse models of influenza A virus pneumonia provide a well-developed experimental system to analyze T cell–mediated immunity. In particular, the T-cell immune response to influenza infection has been well characterized in C57BL/6 (B6,H2^b) mice. While influenza infection of mice does not precisely replicate the natural infection in

human, avian, or other vertebrate species, the availability of reagents and genetically modified mouse models has enabled extensive analysis of the cellular immune response. Emerging evidence indicates that findings from mouse studies are pertinent to immunopathology in human disease. In the BL/6 model, virus is cleared 10 days after infection, with no indication of persistent antigen or viral RNA (*2*). Recovery or prevention of influenza relies on targeting both innate and adaptive responses to the respiratory tract mucosa.

CD8+ T-cell Response to Influenza

Much of the current knowledge on murine CD8+ T-cell responses to influenza has come from analyzing the response to challenge with the HKx31 (H3N2) and PR/8 (H1N1) influenza viruses. A role for CD8+ T cells in protective immunity has been discerned from studies citing delayed influenza virus clearance in CD8+ T cell–deficient mice (*3*,*4*). Furthermore, CD8+ T cells can promote recovery from otherwise lethal secondary viral infections in mice that lack mature B cells or antibodies (*5*,*6*), and cloned influenza-specific CTLs can passively transfer protection (*7*). Despite a seemingly protective role for CD8+ T cells, vaccination with dominant influenza determinants in either a vector or in a recombinant form is only mildly protective (*8–10*). Moreover, in a T cell–receptor transgenic mouse model, devoid of antibodies, influenza-specific CTL can either contribute to survival or exacerbate lethal influenza pneumonia (*11*). This study highlights the need to understand the many facets of the immune response to influenza.

The influenza A virus–specific CD8+ T-cell response has been characterized by using intracellular cytokine staining and MHC class I tetramer labeling. These techniques have enabled each phase of the response to be tracked. After intranasal infection, priming, activation, and expansion of naive influenza-specific CD8+ T cells occur in the draining mediastinal lymph node 3–4 days after infection (*12*,*13*). The antiviral capacity of these virus-specific CD8+ cells is strongly dependent on their ability to migrate and localize to the lungs and infected airway epithelium (*14*), where they appear 5–7 days after infection (*15*). Because viral replication is confined to cells in the respiratory epithelium (*16*,*17*), CD8+ T cells exert their effector functions at this site, producing antiviral cytokines and lysing target cells presenting viral determinants for which they bear a specific T-cell receptor. Lysis of infected epithelial cells is mediated by exocytosis granules containing perforin and granzymes (*18*,*19*). The release of perforin and granzymes from influenza-specific CTLs is tightly regulated, occurring shortly after activation at or near the contact point between CTLs and the infected target cell (*18*).

Influenza-specific CD8+ T cells recognize multiple viral epitopes on target cells and antigen-presenting cells. The HKx31 and PR8 strains share 6 internal genes derived from PR8 that are processed to generate peptides recognized by influenza-specific CD8+ T cells. The primary response to either strain is dominated by CD8+ T cells' recognition of 2 determinants, the nucleoprotein ($NP_{366-374}$, $H2D^b$) (*20*) and the acid polymerase ($PA_{224-233}$, $H2D^b$) (*21*). A similarly low proportion of CD8+ T cells recognizes 4 other determinants: the basic polymerase subunit 1 ($PB1_{703-711}$, $H2K^b$), nonstructural protein 2 ($NS2_{114-121}$, $H2K^d$), matrix protein 1 ($M1_{128-135}$, $H2K^b$), and a protein derived from an alternative open reading frame within the PB1 gene ($PB1\text{-}F2_{62-70}$, $H2D^b$) (*22*). The subsequent memory populations appears to be stable; $D^bNP_{366-374}$ and $D^bPA_{224-233}$ CD8+ memory cells are still detectable ≥570 days after initial infection (K. Kedzierska and J. Stambas, unpub. data).

Secondary influenza-specific CTL responses arise ≈2 days faster than the primary response, with a greatly increased level of activity. Depletion of CD8+ T cells reduces the capacity of primed mice to respond to influenza infection, which indicates a role for CD8+ T cells in the protective secondary response. Prime and challenge experiments can be conducted with HKx31 and PR/8 as all of the recognized epitopes are derived from internal proteins. Furthermore, cross-reactive neutralizing antibodies are avoided because HKx31 and PR/8 express different surface HA and NA or proteins. Despite a similar magnitude to $D^bPA_{224-233}$ in the primary response, $D^bNP_{366-374}$-specific CD8+ T cells dominate the secondary response to HKx31→PR/8 challenge, accounting for up to 80% of the influenza-specific CD8+ T cells. This dominance is maintained in the memory population; the numbers of NP-specific CD8+ T cells exceed all other quantified influenza-specific CD8+ T-cell populations (*23*). Despite the NP dominance, CD8+ T cells specific for the other 5 determinants can still be isolated after secondary challenge, albeit at low frequency.

Conservation of these 6 internal genes and persistence of the corresponding antigen-specific CD8+ T cells makes these genes an attractive target for vaccine therapies. However, although cell-mediated immunity can promote viral clearance, it does not provide sterile resistance because, unlike humoral immunity, it cannot prevent infection of the host cells. In humans, the level of influenza-specific CTLs correlates with the rate of viral clearance but not with susceptibility to infection or subsequent illness (*24*). Despite this limitation, vaccines that promote cell-mediated immunity may be a favorable option to fight potentially lethal, highly pathogenic influenza strains.

CD4+ T cell–specific Responses to Viruses

In contrast to the body of literature that has characterized the role of CD8+ T cells specifically in models of influenza infection, relatively little is known about the role of CD4+ T cells as direct mediators of effector function. That CD4+ T-cell help is central to adaptive immunity is well established, but few antigen-specific systems have been developed to dissect the role of CD4+ T cells in a viral infection. While knowledge of CD8+ T-cell antigen-specific responses has increased substantially in the past several years as a result of tetramer technology, these reagents have been more difficult to develop for the CD4+ subset. Further, identification of CD4+ T cell–specific epitopes has been less successful for a variety of pathogens. For instance, in influenza, the CD8+ restricted epitopes have all been largely identified for some time, particularly in the BL/6 model system; in contrast, only very recently have confirmed CD4 epitopes been found, and they are much more poorly characterized (*25*).

Still, a substantial amount of work has been done with various knockout, depletion, and cell-transfer models to investigate the role of CD4+ T cells in primary, secondary, and memory responses to influenza infection in the mouse model (*26*,*27*). Controversy still exists in the field, and an antigen-specific system would help address it, but certain findings appear to be consistent across different experimental systems (*28*).

In the primary response, CD4+ T cells are not required for expansion or development of functional CD8+ CTL (*27*,*29*), which may in part result from the ability of influenza virus to directly activate dendritic cells, aiding in the development of CD8+ responses that substitute for functional CD4+ T cells (*30*). Similarly, in the case of a murine γ-herpesvirus, the lack of CD4+ T cells can be compensated for by the addition of anti-CD40 stimulation (*31*). In mice in which both the CD4+ T-cell and B-cell compartments were defective, the primary CD8+ T-cell response to influenza appeared to be stunted in terms of recruitment and expansion (vs. mice in which B cells alone were knocked out); the remaining CD8+ T cells had a robust level of functionality as assayed by IFN-γ intracellular cytokine production (*27*). The defect in the CD8+ T-cell primary response was less obvious in mice with intact B cells, though viral clearance was delayed. Still, not until the secondary and memory responses are examined can the dramatic effect of CD4+ T-cell deletion be observed.

In multiple systems, a defect of CD8+ T-cell secondary and memory responses have been observed when the primary response lacks CD4+ T cells (*26*,*32*,*33*). In influenza, a dramatic drop was observed in the size and magnitude of the recall response to secondary infection. The rate of viral clearance was also slowed considerably, beyond the degree seen in the primary response. Similarly, in the *Listeria monocytogenes* model system, the primary response was largely intact, while the long-term memory response was defective (*34*). In mice that lacked CD4+ T cells during the primary response, the memory pool of CD8+ T cells was initially similar in size and functionality to that seen in wild-type mice but began to decline after longer intervals, leading eventually to the recrudescence of the infection. Secondary challenge also demonstrated a reduced antigen-specific CD8+ T-cell compartment.

In the influenza model, although the draining lymph node and spleen CD8+ responses were defective in secondary infection of CD4+ T cell–deficient mice, the CD8+ T-cell responses in bronchoalveolar lavage were equivalent to those seen in wild-type mice (*29*). This finding implies that the high levels of activation and inflammation, in large part mediated by innate immune effectors at the site of infection, were capable of providing the right maturation milieu to expand the response to wild-type levels; this finding suggests CD4+ T cell–specific help is not required at the site of the pathologic changes, at least when the infection induces a high level of other immune stimulation, though it is essential in the lymphoid organs in the generation and maintenance of memory.

A role for CD4+ T cells as effectors has been found in a number of other systems, including the mouse γ-herpesvirus model (*35*) and in HIV-infected humans (*36*,*37*). In these studies, CD4+ T cells contribute to infection control by supplementing their helper role with cytotoxicity. In the case of the γ-herpesvirus, the effector CD4+ population was important only in immunoglobulin –/– μMT mice, while the HIV studies were conducted in infected (and presumably immune-irregular) patients. However, effector CD4+ T cells have been found in multiple stages of the disease and in long-term patients whose disease is not progressing because viral replication is controlled. Finally, a recent report demonstrated a similar cytotoxic CD4+ T-cell effector population in protozoan-infected cattle (*38*).

Relatively few established mouse models are available for studying the CD4+ response to influenza virus. On the IA^d BALB/c background, an HA epitope was discovered, and a transgenic mouse was developed to analyze specific responses (the HNT model) (*39*). This model has been extremely useful for studying several aspects of CD4+ biology in influenza infection, particularly in regards to aging and the development of primary responses leading to acute memory (*39*). Several investigators have introduced external epitopes in influenza to follow CD4+ T-cell responses in defined systems. These include the hen egg lysozyme p46–63 sequence (*40*) and the ovalbumin 323–339 (OT-II) epitope inserted into the NA stalk of WSN influenza virus (*41*). We have inserted the OT-II epitope into the HA of the PR8 H1N1 virus and the X31

H3N2 virus. In contrast to the robust responses achieved with CD8+ T-cell epitopes and transgenics, the CD4+ T-cell responses seem relatively weak (unpub. data). Other naturally occurring epitopes have similarly low frequencies after infection (*25*). The antigen-specific CD4+ response may not develop the dramatic immunodominance hierarchies that are well-known for CD8+ T cells and may be directed at many epitopes, more than are seen in the more-delimited CD8+ T-cell response. Much work needs to be done before this conclusion is certain, and examples of respiratory infections in mice produce robust and dominant responses toward individual class II epitopes (*42*).

Cell-mediated Protection against Highly Pathogenic Influenza

Highly pathogenic H5N1 influenza emerged in 1997, followed by several waves of infection from 2002 until now (*43*). The viruses have been remarkably virulent in multiple animal models, including mice, but little work has been done to characterize the protective immune responses toward H5N1 viruses. A series of reports has shown strong protection toward other highly pathogenic viruses mediated by cellular responses, in the absence of neutralizing antibody. Antibody-deficient mice infected with a mild, passaged strain of an H3N2 virus were more likely to survive than naive controls when challenged with a highly pathogenic H3N8 duck virus compared to naive controls (*44*). A double-priming protocol provided increased protection from a lethal H7N7 challenge, which correlated with an increased pool of cross-reactive antigen-specific CD8+ T cells (*45*). In both these cases, the initial phase of infection and viral growth seemed similar to that occurring in immunologically naive mice, but a rapid decrease in viral titers occurs after a few days of infection.

Since the emergence of the H5N1 viruses, concern has arisen that the biological activity of these viruses, including their diverse tissue tropism in a number of animal models, may influence the ability of immune responses to control infection. Furthermore, some pathology associated with these viruses has been attributed to extremely high levels of inflammatory cytokines produced in response to infection, which suggests a negative role for immune responses. However, the few studies that have been performed have shown promising results for the potential of cell-mediated responses to contribute to the control of infections. A prime-challenge protocol using an H9N2 isolate with 98% homology to the internal genes of the A/HongKong/156/97 H5N1 protected against the otherwise lethal challenge (*46*) with a virus with a highly cleavable H5, a characteristic of all the pathogenic H5 viruses. The priming protocol generated significant CTL activity directed at the NP and PB2 proteins.

Our own work has indicated a similar ability of cell-mediated immunity to protect against virulent H5N1 challenge. In a preliminary experiment, we primed mice with the H1N1 PR8 strain and the H3N2 X31 strain followed by a challenge with A/Vietnam/1203/2004, one of the most lethal H5N1 viruses, which causes severe pathologic changes, even in ducks. While 9 of 10 naive mice died, 9 of 10 primed mice survived past day 10 of infectious challenge and recovered substantial weight (Figure). The fact that both groups lost weight indicated protection was occurring by delayed cell-mediated responses, rather than by the "immediate" cross-protective antibody response.

Cell-mediated Vaccine for Highly Pathogenic Influenza?

Despite the systems currently in place for manufacturing and distributing an influenza vaccine, pandemic influenza will require a substantially different approach. The standard influenza vaccine given during the infectious season is made from a reassortant seed strain containing the HA and NA of the circulating virus with the internal genes of a vaccine strain, usually PR8. The seed strain is grown in eggs and is formaldehyde inactivated. This strategy does not prime strong CD8 CTL responses, but it is effective in providing antibody-mediated protection to closely homologous strains (*47*).

One drawback to this approach is the length of time required to develop a seed strain, amplify it, and manufacture it into distributable vaccine. In the case of a potential influenza pandemic, the delivery of vaccine on this schedule would not prevent the spread of the epidemic in many countries. Furthermore, antigenic drift can occur between the original selection of the seed strain and circulating viruses before the vaccine is ready for distribution (*48*).

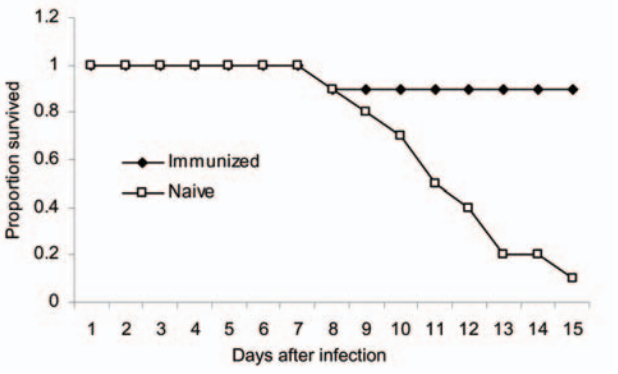

Figure. Apparent cell-mediated protection against highly pathogenic H5N1 influenza virus. Mice (10 in each group) were immunized by intraperitoneal injection of PR8, followed by intraperitoneal injection 4 weeks later of X31. Four weeks after the second immunization, immunized or naive mice were infected with 300 mouse lethal dose 50% of A/Vietnam/1203/2004.

This problem was faced recently in a nonpandemic situation in 2003 and 2004 when the circulating Fujian strain of H3N2 influenza had drifted from the vaccine strain (*49*). While the Fujian strain was predicted to be circulating at the time of vaccine delivery, a recombined seed strain could not be isolated in time for vaccine production. Although the ensuing influenza season was not as severe as initially feared, the situation highlighted a problem with the current vaccine strategy. Evidence of antigenic drift is already evident in the most recent outbreaks of H5N1 (*48*).

Several groups have developed reverse genetics–based methods that could speed the production of seed viruses as well as proposals for growing viruses in cell culture rather than in embryonated chicken eggs, which would allow for a much faster scale up in response to an epidemic (*50*). These technologies have not been approved yet for human use, though trials are underway.

Even if the development of recombinant seed strains by reverse genetics becomes standard over the next few years, questions remain about how effective the current formaldehyde-inactivated seed strain strategies would be against pandemic strains, particularly the currently circulating H5N1 strains. Assuming that seed strains could be produced rapidly, several weeks would be required to manufacture a relevant number of doses of vaccine. To address this concern, several governments have been stockpiling vaccines based on H5N1 viruses that have been circulating over the last few years. While these vaccines may provide some protection, substantial evolution and antigenic drift seem to be occurring, rendering the stockpiled strains less and less useful (*48*).

An approach based on conserved cellular epitopes within the internal genes has the advantage of subverting all of these issues. While cellular immunity is not sterilizing, it prevents illness and death in animal models (*3*). Common and immunodominant epitopes among circulating nonavian strains have been identified, and many of the same models and algorithms can be used to make predictions against the pathogenic strains (*51*). Mouse models are now available that have human leukocyte antigen (HLA) alleles, and they appear to recapitulate human epitope use. As described earlier, protection against death from highly pathogenic viruses has been shown in multiple systems. Cross-protective cell-mediated immunity has been found in birds for circulating chicken H5N1 and H9N2, both of which have been suggested as potential human pandemic strains (*52*). The notion of a "universal" vaccine for highly pathogenic strains is attractive.

Antigenic drift due to immunologic pressure is also a concern with a CD8- or CD4-based vaccine approach. Reports have suggested that CD8+ epitopes under pressure will mutate to escape protective immunity (*11*). The mutation of an NP epitope that binds HLA-B35 present in strains of virus from the 1930s through the present indicates that even in nonpandemic years, immunologic pressure from cross-protective CD8+ T cells is enough to drive the evolution of the virus (*53*). In contrast, though, other dominant epitopes do not appear to be under the same pressure (*54*).

Several human peptide epitopes that have been described and characterized show evidence of remarkably little mutation over many generations of viral evolution. In the most recent outbreaks of H5N1 virus, some of these peptides are conserved in viruses isolated from human patients (Table). The conservation of so many peptides from such distantly related viruses suggests that they may be less susceptible to antigenic drift than the HA and NA glycoproteins. Vaccines that promote strong memory CTL activity toward these peptides and MHC, in combination with the antibody-based approaches already underway, could help prevent pandemic influenza. This approach could potentiate immunologic pressure on the vaccine-targeted epitopes, but an immunization strategy that targets a large number of epitopes along with the natural restriction on epitope structure due to viral function should mitigate this effect. Some evidence shows that highly conserved CTL epitopes are restricted from mutation by viral structural requirements. Given the large number of influenza

Table. Conservation of human NP and M1 epitopes between H1N1 PR8 and 3 human isolates of H5N1 viruses (A/Hong Kong/156/1997, A/Hong Kong/213/2003, and A/Vietnam/1203/2004)*

Epitope	HLA restriction	PR8 sequence	Conservation
NP 383–391	B*2705	SRYWAIRTR	3/3 identical
NP 418–426	B*3501	LPFDRTTIM	0/3 identical
NP 44–52	A*01	CTELKLSDY	2/3 identical (156 Y9Q)
NP 265–273	A*03	ILRGSVAHK	3/3 identical
NP 188–198	A*1101	TMVMELVRMIK	3/3 V7I mutation
NP 380–388	B*08	ELRSRYWAI	3/3 identical
NP 174–184	B*2705	RRSGAAGAAVK	2/3 identical (156 V10I)
M1 58–66	A*0201	GILGFVFTL	3/3 identical
M1 27–35	A*03	RLEDVFAGK	2/3 mutated (1203, 213 both R1K)
M1 13–21	A*1101	SIIPSGPLK	3/3 identical

*All 3 isolates were compared to the mouse-adapted PR8 strain and differences are reported. Sequences obtained from the Influenza Sequence Database (*55*). NP, nucleoprotein; HLA, human leukocyte antigen.

viruses sequenced over time, we should be able to make reasonable assumptions about the identity of these epitopes in MHC-diverse populations and focus on how to facilitate the development of strong immune responses toward them.

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Dr Thomas works in the Department of Immunology at St. Jude Children's Research Hospital in Memphis, Tennessee. His primary research involves the use of reverse genetics–engineered influenza viruses to understand the dynamics of CD8+ and CD4+ T-cell responses.

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Address for correspondence: Peter Doherty, St. Jude Children's Research Hospital - Immunology, 332 N Lauderdale St, Mailstop 355, Memphis, TN 38105, USA: fax: 901-495-3107; email: Peter.Doherty@stjude.org

Vaccines and Antiviral Drugs in Pandemic Preparedness

Arnold S. Monto*

While measures such as closing schools and social distancing may slow the effects of pandemic influenza, only vaccines and antiviral drugs are clearly efficacious in preventing infection or treating illness. Unless the pandemic strain closely resembles one already recognized, vaccine will not be available early. However, studies can be conducted beforehand to address questions concerning vaccine dose, frequency of inoculation, and need for adjuvants. In contrast, antiviral drugs, particularly the neuraminidase inhibitors, will be effective for treatment and available if stockpiling takes place. Special questions need to be answered if a highly lethal virus, such as influenza A (H5N1), produces the pandemic. Both vaccines and antiviral drugs will be required for a coordinated strategy.

Prevention of influenza, particularly during a pandemic, may be attempted by many measures, such as closing schools, using facemasks, and keeping infected persons away from those susceptible, now termed social distancing. However, none of these measures are of clear value in preventing infection, even if they could be accomplished. A principal reason little effort has been made to determine their usefulness in the interpandemic period is the usual availability of vaccine, which is of known value in prevention. Thus, few studies have been undertaken. Similarly, symptomatic therapy is possible and perhaps appropriate in treating milder illnesses. Antimicrobial drugs are necessary when bacterial complications occur. However, antiviral drugs are specific and can not only prevent infection but also treat illness (*1*).

A pandemic virus will likely spread so rapidly from the source that vaccine availability may be delayed for months after major outbreaks begin. In addition, much of the population will be totally susceptible. We will likely not be able to prepare stockpiles of virus concentrates well matched with the pandemic strain for vaccine production before the strain has actually shown itself. In contrast, antiviral drugs, particularly the neuraminidase inhibitors (NAIs), will be effective against any pandemic virus, and stockpiling is possible (*1*). However, supplies will likely be limited, even with a relatively large stockpile, and may well be exhausted without careful planning before vaccine is available.

*University of Michigan School of Public Health, Ann Arbor, Michigan, USA

Vaccines: Needs and Priorities in the Prepandemic Phase

Key to the ability to have vaccines ready is early detection of the pandemic virus. Improved surveillance networks are vital for this purpose. While the specific variant that emerges will probably be different antigenically from any recognized, much can be learned by studying the known variants of likely subtypes. An example of what needs to be done before the pandemic is the concerted evaluation in 1976 of a virus variant thought to have pandemic potential (*2*). The swine influenza virus, detected in humans in that year, was viewed as a pandemic threat. Because the pandemic never occurred, researchers had time to complete a large range of pediatric and adult studies. We learned that those who had no previous experience with that subtype needed to be vaccinated twice with a split preparation. The whole-virus vaccine then commonly used could not be given to those persons without frequent systemic reactions, but the whole-virus vaccine was more immunogenic and might be acceptable if rapid response was desired. In persons previously exposed to the influenza virus subtype, the whole-virus vaccine was much less reactogenic and appeared more immunogenic than the split product (*3*,*4*). These observations still have relevance in the current situation.

Similar studies need to be carried out now on all subtypes of pandemic potential. However, we cannot do so without choosing priorities, given restrictions of time and resources. Choices must be made on the basis of historic and current observations. At one time, a closed, fixed cycle of type A subtypes was thought to exist, with one following the other, each producing a pandemic (*5*). This theory

predated molecular analysis of the hemagglutinin of the viruses and was based as a classification system derived from their epidemiologic characteristics. As shown in the Table, the concern that swine influenza would appear in 1976 was supported by seroarcheology, evidence in serum samples collected before, for example, 1968 that an A (H2) and A (H3) virus had previously circulated (*6*). Few currently believe this theory in its entirety, since it would require that a subtype remain undetected in a host, perhaps in humans, for a long period of time. However, the determination, using molecular techniques unavailable until well after the pandemics had occurred, that the A (H2N2) and A (H3N2) viruses were reassortants between previous human and avian strains suggested a different origin for these viruses (*7*,*8*). The avian predecessors of these 2 new viruses were not highly pathogenic, and the resultant pandemics showed a typical U-shaped death rate, highest in the very young and old. The 1918 virus had a different derivation and was apparently not a reassortant but a mutant. It also had an avian origin, but the progenitor virus has not yet been identified, so its pathogenicity in birds is unknown (*9*). However, its epidemiologic signature in humans was high case fatality in young adults (*10*).

The question, then, based on this evidence, is which viruses should be studied to prepare a vaccine to control the next pandemic? Will type A (H2N2) return, in keeping with the recycling theory? Much of the population will now be susceptible. Type A (H9N2), a less pathogenic avian virus, has transmitted occasionally to humans, with little or no further transmission, but has not produced disease with high case fatality (*11*). The highly pathogenic type A (H5N1) virus is at the top of the list of potential pandemic threats. This virus, if it becomes adapted for human transmission without a reduction in virulence, could result in a pandemic far worse than 1918, also involving healthy, younger persons (*12*,*13*). Other viruses, such as the A (H7) highly pathogenic avian strains, including A (H7N7), which infected humans in the Netherlands, and A (H7N3), which spread extensively in western Canada, can also be considered candidates but are not as high on the list since fewer transmissions to humans and less clinical disease have been seen (*14*,*15*).

Prepandemic Vaccine Evaluation

Scientific questions that have been raised concerning the various priority potential pandemic viruses are different, depending on the specific subtypes. The goal in all cases is production of an immune response with the least amount of antigen, so that more doses can be available. Perhaps the simplest situation is that of A (H2N2), a known quantity, because of its presence from 1957 to 1968, in terms of immune response, population likely to be infected, and expected disease characteristics. Also, that virus presents the fewest issues about vaccine production, for the same reasons. However, the basic question relates to producing the best immune response with the least amount of antigen and avoiding if possible the need for a second injection, which would use additional antigen and delay production of protective immunity. One approach, already studied, is to leave the harvested virus particles intact, the modern equivalent of the whole-virus vaccines evaluated in 1976 (*16*). In persons without prior infection with this virus, 1 injection of as little as 3.8 μg with alum, a widely used adjuvant, produced some antibody response, as determined by the hemagglutination-inhibition (HAI) test, traditionally used to assess protection afforded by inactivated vaccines. A second injection produced high titers. Positive features of this approach are that vaccine could be produced more quickly, and antigen would be spared. A possible negative feature would be reactogenicity in children. However, we do not know whether, with modern purification methods, these vaccines would have the reactogenicity of those produced in 1976. Less work has been done with avian influenza, A (H9N2), but similar approaches might be used with these nonhighly pathogenic avian viruses (*16*,*17*).

Table. Influenza A subtypes in humans

Year of recognition	Old terminology	Molecular antigenic terminology
1889		H2*
1902		H3*
1918	Swine influenza	H1N1
1932	A0	H1N1
1947	A prime	H1N1
1957	Asian	H2N2
1968	Hong Kong	H3N2
1976	Swine	H1N1†
1977	Russian	H1N1

*Unknown N subtype.
†Limited human-to-human transmission.

The highly pathogenic A (H5N1) virus presents many more problems in vaccine development and evaluation. The first one, already solved, involves removal of the molecular motif of high pathogenicity, the multibasic cleavage site, from the hemagglutinin. The virus is then reassembled by using reverse genetics, but on a background of the high-growth type A virus, PR8 (*18*). Producing vaccine by using this engineered virus can then proceed without high-level containment. However, we know from previous work with a less pathogenic influenza, A (H5N3) that antibody response to this avian subtype is not good and that adjuvants and multiple doses are required (*19*,*20*). The A (H5N3) vaccine was given to only small numbers of healthy adults. Response did not occur in persons given ≤30 μg of antigen alone but did in persons given the antigen with the MF-59 adjuvant. However, after 16 months, essentially no antibody was seen even in those

who received the vaccine with adjuvant. On revaccination with the same preparation, persons previously given the vaccine with adjuvant had an anamnestic response, while persons given the unadjuvanted vaccine again had a poor response. Measuring and evaluating the meaning of the antibody response to some avian viruses is also an issue. Even infection with the A (H5N1) virus does not produce a good HAI antibody response; the antibody needs to be detected with a neutralization test (*21*). Similarly, neutralization testing is necessary to detect response to vaccine; however, a specific level of HAI antibody has been associated with protection, but no similar correlate of neutralization antibody has yet been developed (*5*).

Further evaluation of dosage and need for booster injection of these vaccines is in process. An international agenda is needed so that the diverse issues will be systematically investigated. Several high-priority vaccines need to be evaluated at various frequencies of administration and dose levels, with and without adjuvants. No single country can do it adequately (*10*). The work has started, especially with the A (H5N1) vaccine produced by reverse genetics, but the research has a long way to go.

Antiviral Drugs: What Can be Done Before the Pandemic

With antiviral drugs, the scientific questions that need to be answered before the pandemic are not as daunting (*13*). Originally, both classes of antiviral drugs were believed to be effective against a pandemic virus. Adamantane action is limited to type A viruses, but all pandemic viruses are type A (*15*). The neuraminidases of many different type A viruses have been evaluated with respect to NAIs, and all have been found susceptible (*1*). As a result, given advance planning so that supplies are available, antiviral drugs can be used early in a pandemic and do not require specific production and formulation. Because they are much less costly than NAIs, adamantanes were part of the overall antiviral strategy (*20*). Having 2 classes of drug increased the amount of antiviral drugs available to stockpile since production limitations are an issue with the NAIs.

Considerable evidence indicates that both classes of drugs work well in prophylaxis against susceptible seasonal influenza viruses and that prophylaxis does not increase resistance. In fact, amantadine prophylaxis has been tested in a pandemic situation, and while efficacy may be reduced in persons with no previous exposure, which seems to increase protection, it is still 70%–80% (*22*). Although no direct comparisons have been carried out with the adamantanes, NAIs appear at least as efficacious. The 2 NAIs, zanamivir and oseltamivir, gave similar results when given daily for 4 or 6 weeks (*23*,*24*). They may be more efficacious in preventing febrile illnesses, although asymptomatic infection often still occurs. This characteristic is actually desirable, since it provides protection against the next wave of the pandemic virus. However, in some cases, infection is prevented completely, so vaccine should be used when available.

In treatment, adamantanes and NAIs diverge in their efficacy. No reliable data on use in pandemics exists, and no head-to-head studies have been carried out. Studies of treatment with amantadine and rimantadine did not allow firm estimates of how much they shortened duration of illness but were sufficient to conclude that they produced more rapid resolution than symptomatic therapy, such as aspirin (*25*). No data suggest that they prevented complications in any population; indeed, recent experimental studies suggest that they do not (*26*). However, the main reason they have never been considered for therapy in a pandemic is that antiviral resistance occurs in ≥30% of those given the drug for treatment and that resistant viruses are fully pathogenic and transmissible (*27*). While resistance occurs when oseltamivir is used in treatment, it is far less frequent than with the adamantanes, and the mutant viruses may be less infectious and transmissible than wild type (*28–30*). This conclusion cannot be viewed as absolute; with high-volume use, which has occurred thus far only in Japan, resistant viruses could begin to circulate. Emergence of resistance has apparently occurred with adamantanes, and the more recent type A (H5N1) virus, as well as some currently circulating seasonal viruses, are not susceptible to this drug class.

Another advantage of NAIs in therapy is their ability to prevent certain complications (*31*,*32*). Some evidence also shows increased efficacy in illnesses that are identified as more severe at onset (*33*). We cannot predict how this efficacy would translate into treatment success in a pandemic, but it encourages using them to treat persons who are recognized early to be more symptomatic.

With ordinary influenza viruses of pandemic potential, such as type A (H2N2) and A (H9N2), treatment success in the interpandemic period would be more likely relevant to the pandemic. Such may not be the case with the type A (H5N1) virus. The virus has evolved since the 1997 Hong Kong outbreak, and some evidence of a systemic infection involving the brain and gastrointestinal tract exists (*12*,*34*). This infection has also been demonstrated in laboratory animals such as ferrets (*35*) and means that the drug may need to reach adequate concentration in these sites, remote from the respiratory tract. Zanamivir is not orally bioavailable and is thus not likely to be useful in treating influenza A (H5N1) infection, although it might play a role in prophylaxis. Oseltamivir, in contrast, is absorbed and metabolized. While human studies of oseltamivir in treatment would be critical now, such studies have been difficult to carry out, since the disease has been occurring in

areas where recognition of the cause is often delayed. We have yet to determine whether the mixed results that have been described with this drug in the limited case reports are due to late treatment or other factors, such as need for higher doses (*13*,*35*). A planned clinical trials network may solve this problem. In the meantime, animal studies are urgently needed to evaluate dosage and duration of therapy, particularly against the Vietnam strain of the A (H5N1) virus. These studies would help guide treatment of human cases until more data are available. Mouse studies have already indicated that, while oseltamivir is effective, it is not as effective when given for 5 days as it was against the 1997 Hong Kong variant of A (H5N1) influenza (*36*). This finding indicates that treatment for 10 days might be necessary, since in the mouse studies, replication resumed after therapy was stopped. The dose may also need to be increased. Studies in ferrets and nonhuman primates would have more relevance to the situation in humans than studies in mice.

Vaccine Activities in the Pandemic

Countries will need to have pandemic plans in place to establish priorities for vaccine use. However, to help refine these decisions once the pandemic begins, epidemiologic- and vaccine-related issues will have to be addressed. The pandemic must be characterized not only in terms of the groups infected but also, more importantly, case fatality in each group. Vaccine supply will be increasing over time, so the question is which groups should get it earlier. Current pandemic planning usually directs vaccine to the groups who traditionally have had the highest death rates, mainly the old and the very young, but this might have to change. If the 1918 pattern repeated itself, or for example, if the A (H5N1) virus produces the pandemic and does not change in virulence or its tendency to infect the young, vaccination priorities would have to be changed radically.

Once the pandemic virus is available, a rapid evaluation will be needed to address questions of dosage, need for adjuvants, and booster vaccination. However, this evaluation will need to be done quickly, especially for regions of the world close to the pandemic origin, so as much work as possible should be done before the pandemic. First, though, a virus for vaccine production will need to be created from the pandemic strain, with appropriate manipulation to make it high yielding. In the process, the molecular and antigenic differences between this virus and those of the same subtype already available will need to be defined. With luck, the pandemic virus may be similar enough to one already studied so that any available concentrates can be used. However, similarity is unlikely because of the antigenic variation of influenza strains within a subtype. Rather than stockpiling, another strategy needs to be considered for vaccines containing a virus such as A (H5N1) for which vaccine development has already begun. That virus can be included in vaccines in use before the pandemic. Although influenza A (H5N1) virus has been evolving, even a poorly matched vaccine might provide some protection, especially against a variant with such high lethality (*37*). Also, if 2 injections of a specific vaccine are necessary, an older vaccine could prime, so that only 1 injection of the new vaccine would be needed. An A (H5N1) vaccine might initially be directed for use in areas such as Southeast Asia, which are experiencing continued avian transmission and occasional spread to humans.

A live, attenuated vaccine would more likely produce antibodies after 1 injection and would have a number of other theoretical advantages over inactivated vaccine in a pandemic. Unfortunately, such a vaccine will not generally be considered for 2 reasons. First, production requires specific pathogen–free eggs and these will be in shorter supply than ordinary eggs. This could change if cell culture could be used. However, the bigger problem involves evaluation before and use early in the pandemic. Since this vaccine virus could reassort, it might introduce the pandemic virus into the population if used too early. The question also arises whether attenuation would be successful with a new and potentially more virulent wild type, a result which could be evaluated in advance in animals (*38*).

Antiviral Drugs in the Pandemic

While supplies of vaccines will increase as the pandemic evolves, antiviral drug supplies will decrease as stockpiles are depleted. The starting level will depend on the amount of stockpiling, based more on economic and policy consideration than science. As with vaccines, planning decisions will be in place to prioritize use during the initial period, which may need to be modified based on epidemiologic characteristics of the outbreak and clinical characteristics of the cases. The key virologic issue will be whether the pandemic strain is susceptible to the antiviral drugs. Most recent planning, since it is focused on the threat of the A (H5N1) virus, has assumed that adamantanes would not be useful. This assumption means that if the disease is systemic and case fatality is high, among the NAIs only oseltamivir would be useful, since it is absorbed (*39*). Given the limited quantities likely to be available, at least in the near future, the drug will have to be restricted to treat those most likely to die or have severe consequences. Careful observation of treatment results will help to determine if the dose and duration of therapy is appropriate. Seasonal prophylaxis uses larger quantities of drug, but possibly limited postexposure use could be feasible. Zanamivir, if available, might find its role in prevention. Infection is likely through the respiratory tract, and given past evidence, the drug could make a major contribution in prophylaxis before vaccine is available. Throughout,

mechanisms need to be in place to monitor antiviral resistance, which might emerge as a problem with extensive use of the drugs. A long-term goal should be to develop new antiviral agents against influenza. The global reliance on basically 1 drug from 1 source cannot be allowed to continue. Other NAIs are available for clinical evaluation, and drugs targeting other phases of influenza viral replication would be especially useful.

Given the threat of a virulent virus such as A (H5N1) and the suggestion that adaptation to transmissibility may occur gradually, the concept has emerged that antiviral drugs may be used to interrupt early, local transmission. The aim would be to prevent spread out of the region of origin, in other words, extinguishing the epidemic at its source (*40*). Transmission models suggest that this strategy will work as long as the R_0 or basic reproductive number is not high (*41*). Thus, this goal seems worthy of consideration on more than a theoretical basis. Models also suggest that the approach might be more likely to succeed with partial immunity in the population (*42*). This immunity could be produced by prior vaccination with a current A (H5N1) vaccine. Practical issues may be of greatest concern, especially the ability to put antiviral prophylaxis in place rapidly in rings around cases. Supplies of oseltamivir are also an issue. Will those countries with stockpiles be willing to share with other countries on the possibility, not certainty, that a pandemic could be avoided?

Conclusion

Major challenges are presented in controlling a pandemic with vaccine and antiviral drugs, particularly one caused by an A (H5N1) virus similar to those currently circulating. Some are specific to the particular intervention, but others are more generic. Long-term needs exist, such as developing innovative technologies for vaccine prevention and designing antiviral drugs to affect different targets. However, immediate attention for vaccines must be directed to a coordinated international approach to vaccine evaluation, paying attention to ways in which the least amount of virus can immunize the largest number of persons. Use of a possibly unmatched A (H5N1) vaccine for priming should be considered, especially in Southeast Asia, or other areas with the most pressing need. In those regions, antiviral strategies need to be evaluated; drug studies in animal models will be necessary, given the sporadic nature of the disease in humans. Overall, developing countries will have limited access to vaccines and antiviral drugs, and their needs must not be forgotten. With marginal healthcare infrastructures, they will suffer the most, whatever the severity of the pandemic.

Dr Monto is professor of epidemiology at the University of Michigan School of Public Health. His major research interests are assessing the efficacy of live and inactivated vaccines in prophylaxis and the neuraminidase inhibitors in therapy of influenza, and working with the United States and international organizations on pandemic preparedness.

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Address for correspondence: Arnold S. Monto, Department of Epidemiology, University of Michigan School of Public Health, 109 Observatory St, Ann Arbor, MI 48109-2029, USA; fax: 734-764-3192; email: asmonto@umich.edu

Making Better Influenza Virus Vaccines?

Peter Palese*

Killed and live influenza virus vaccines are effective in preventing and curbing the spread of disease, but new technologies such as reverse genetics could be used to improve them and to shorten the lengthy process of preparing vaccine seed viruses. By taking advantage of these new technologies, we could develop live vaccines that would be safe, cross-protective against variant strains, and require less virus per dose than conventional vaccines. Furthermore, pandemic vaccines against highly virulent strains such as the H5N1 virus can only be generated by reverse genetics techniques. Other technologic breakthroughs should result in effective adjuvants for use with killed and live vaccines, increasing the number of available doses. Finally, universal influenza virus vaccines seem to be within reach. These new strategies will be successful if they are supported by regulatory agencies and if a robust market for influenza virus vaccines against interpandemic and pandemic threats is made and sustained.

Influenza virus vaccines were first developed in the 1940s and consisted of partially purified preparations of influenza viruses grown in embryonated eggs. Because of substantial contamination by egg-derived components, these killed (formaldehyde-treated) vaccines were highly pyrogenic and lacking in efficacy. A major breakthrough came with the development of the zonal ultracentrifuge in the 1960s (invented by Norman G. Anderson) (*1*). This technology, which originated from uses for military purposes, revolutionized the purification process and industrial production of many viruses for vaccines. To this day, it remains the basis for the manufacturing process of our influenza virus vaccines.

Current influenza virus vaccines consist of 3 components: an H1N1 (hemagglutinin [HA] subtype 1; neuraminidase [NA] subtype 1), an H3N2 influenza A virus, and an influenza B virus. Specifically, the 2005–2006 vaccine formulation is made up of the A/New Caledonia/20/99 (H1N1), A/California/7/2004 (H3N2), and B/Shanghai/361/2002 viruses. Changes in the HA of circulating viruses (antigenic drift) require periodic replacement of the vaccine strains during interpandemic periods. The World Health Organization publishes semiannual recommendations for the strains to be included for the Northern and Southern Hemispheres (*2*). To allow sufficient time for manufacture, in the United States the US Food and Drug Administration (FDA) determines in February which vaccine strains should be included in the following winter's vaccine. Unfortunately, FDA recommendations are not always optimal. For example, in 2003 FDA rejected the use of the most appropriate H3N2 strain, A/Fujian/411/2002, and instead again used the same strain as in the 2002 formulation. This decision was made primarily because the A/Fujian/411/2002 strain had first been isolated in Madin Darby canine kidney (MDCK) cells rather than in embryonated eggs. Use of MDCK cells for virus isolation is not allowed by FDA's rules, which do not yet encompass advanced technologies or scientifically sound purification procedures based on limiting dilutions or cloning with DNA. Because of this bureaucratic roadblock, the H3N2 component of the 2003–2004 influenza virus vaccine was antigenically "off" and showed suboptimal efficacy. One hundred fifty-three pediatric deaths were associated with influenza infections during the 2003–2004 season in 40 states, whereas only 9 such deaths had been reported in the following season (*3*). Also, because the cumbersome classical reassortment technique used for preparing the appropriate seed strains makes the yearly process of manufacturing influenza virus vaccines unnecessarily lengthy, new variants first appearing early in the season are rarely considered for the vaccine formulation of the following winter.

Currently Licensed Influenza Virus Vaccines

Most influenza virus vaccines used in the United States and Europe consist of embryonated egg-grown and formaldehyde-inactivated preparations, which, after purification, are chemically disrupted with a nonionic detergent

*Mount Sinai School of Medicine, New York, New York, USA

(for example, Triton X-100). The split virus preparations show lower pyrogenicity than whole virus vaccines. In general, 1 dose for adults contains the equivalent of 45 μg HA (15 μg HA for each of the 3 antigenic components). This dose is approximately the amount of purified virus obtained from the allantoic fluid of 1 infected embryonated egg. If 100 million doses of killed influenza virus vaccine are prepared, the manufacturer has to procure 100 million embryonated eggs. Clearly, this manufacturing process is dependent on the timely availability of embryonated eggs and the vaccine seed strains to be used in a particular season. Most of these prototype seed strains are provided to the manufacturers by government agencies, which create high-yielding strains through classical reassortment with a high-yielding laboratory strain, A/PR/8/34, following the procedures designed by Kilbourne (*4*). Unfortunately, only (high-yielding) influenza A viruses can be made in this way, and even with the A types, the 6:2 reassortants (HA and NA from recently circulating strains and the remaining 6 genes from A/PR/8/34 virus) are sometimes not easily obtained. This time-consuming process of reassortment is then followed by repeated passaging of the strain in embryonated eggs to allow for egg adaptation and growth enhancement. Influenza B virus prototype strains with good growth characteristics are usually obtained by direct and repeated passaging in embryonated eggs without attempting to generate reassortants. Although the manufacturing process is time-consuming, these killed influenza A and B virus vaccines are the workhorses for vaccination against influenza and have been shown time and again to be highly effective.

The second major class of viral vaccines consists of live viruses. The only FDA-licensed product against influenza is the cold-adapted attenuated vaccine. It is based on work originally done by Maassab's laboratory (*5*) and later by Murphy and colleagues (*6*). Influenza virus was passaged at 25°C in tissue culture (chicken kidney cells) and in embryonated eggs. This modified Jennerian approach resulted in a cold-adapted, temperature-sensitive, and highly attenuated master strain. The annually updated vaccine strains are generated in the laboratory by reassortment with viruses more closely related to the currently circulating ones. The resulting vaccine strains (both A and B types) are 6:2 reassortants with the 6 nonsurface protein genes derived from the cold-adapted master strains and the HA and NA from circulating A and B viruses, reflecting the changing antigenicity. These cold-adapted influenza virus vaccines are easily administered by nasal spray. They induce local mucosal neutralizing immunity and cell-mediated responses that may be longer lasting and more cross-protective than those elicited by chemically inactivated (killed) vaccine preparations. Vaccine efficacy in vaccine-naive children 6 months to 18 years of age is high (range 73%–96%). In children revaccinated for a second season, vaccine efficacy climbs to 82% to 100% (*7*).

Need for Improvement?

Despite the obvious efficacy of both killed and live influenza virus vaccines, there is room for new developments. Among the critical issues in developing new and better vaccines are the following: price per dose, speed of production, ease of production, choice of substrates to grow the virus in or to express viral antigens, cross-protection for variant strains, efficacy in general and in immunologically naive populations, safety, and acceptance by the regulatory agencies and the public.

New Adjuvants

Most of the current inactivated influenza virus vaccines do not contain an adjuvant. To stretch the available supply, antigen-sparing adjuvant approaches should be considered (*8*). Alum is an adjuvant that has been approved by the FDA for use in several vaccines. MF59, a proprietary adjuvant from Chiron (Emeryville, CA, USA), has also been successfully used in several countries (other than the United States). If, under adjuvant conditions, a fifth or a tenth of the antigenic mass currently present per vaccine dose (45 μg of HA protein) would suffice to stimulate an adequate protective response, a big supply problem would be solved.

Many adjuvants are now under investigation. Liposome-like preparations containing cholesterol and viral particles (immune-stimulating complexes) have been successfully used in mice (*9*) by subcutaneous and intranasal administration. Another adjuvant strategy involves the use of heat-labile *Escherichia coli* toxin complexed with lecithin vesicles and killed trivalent influenza virus preparations for intranasal administration (*10*). Although this specific vaccine has been withdrawn because of Bell's palsy cases associated with its administration, similar approaches may become more acceptable in the future if these safety issues can be resolved. Much work is also currently being conducted on synthetic adjuvants, such as synthetic lipid A, muramyl peptide derivatives, and cationic molecules (*11*). Also, Ichinohe et al. showed that poly (I:C) is a promising new and effective intranasal adjuvant for influenza virus vaccines (*12*).

Genetically Engineered Live and Killed Influenza Virus Vaccines

As indicated, current FDA-licensed influenza vaccines are based on technologies developed in the 1960s and earlier. Through the breakthrough of reverse genetics techniques (*13–15*), infectious influenza viruses from plasmid DNAs transfected into tissue culture cells can now be rescued. This technology permits the construction of high-

yield 6:2 seed viruses by mixing the 6 plasmid DNAs from a good-growing laboratory strain with the HA and NA DNAs obtained by cloning relevant genes from currently circulating viruses. Thus, within a 1- to 2-week period, the appropriate seed viruses could be generated for distribution to the manufacturers. The backbones of the 6:2 recombinant viruses could be prepared, tested, and distributed in advance. Similar approaches can be envisioned for the manufacturing of live, cold-adapted influenza virus vaccines. In this case, the backbone would consist of the 6 genes of the cold-adapted master strain. Again, the HA and NA of the currently circulating strains would be cloned and used for rescue in the plasmid-only reverse genetics system. Such an approach would have several advantages over the present manufacturing process. First, it would dramatically accelerate the timeframe for obtaining seed viruses for annual production and thus allow more time to select the appropriate antigenic seed strains. Second, it would standardize the seed viruses to be used. Regulatory agencies do not insist on a sequenced product to be given to humans but instead allow only partially characterized products for annual immunization. Third, DNA cloning may eliminate any adventitious agents present in the throat washings of the original isolate. Finally, in the case of the current highly pathogenic H5 strains, viruses with that HA (containing a multibasic HA1/HA2 cleavage site) kill embryonated eggs, making it difficult to use eggs as growth substrate. Also personnel involved in manufacturing those vaccines might be in danger of becoming infected. Thus, the HA of these virulent strains will need to be modified. Removal of the basic cleavage peptide by reverse genetics results in a virus that is attenuated for embryonated eggs, thus allowing high yields to be attained. Modification by reverse genetics results in a product that is easier to manufacture and safer to handle (this includes safety considerations for all persons working with the virus).

Live Influenza Virus Vaccines with Altered Nonstructural Protein 1 Genes

The ability to site specifically engineering changes in the influenza virus genome also allows us to consider novel vaccine approaches. We have demonstrated that the nonstructural protein 1 (NS1) of influenza viruses has interferon antagonist activity (*16*). Influenza viruses that lack NS1 cannot counter the interferon response of the host. Thus, infection of cells with a virus that lacks NS1 results in the induction of interferon and blockage of virus replication. When truncations are made in NS1, viruses are generated with an intermediate activity, which enables them to replicate in the host and also to induce an interferon response. By engineering a virus with intermediate virulence and ability to induce interferon, one can construct ideal influenza virus vaccines that are both attenuated and highly immunogenic (*17–20*). Interferon appears to be an excellent adjuvant that enhances production of immunoglobulins and contributes to the activation of dendritic cells required for antigen presentation (*21–23*). We thus believe that, per virus particle made or antigen molecule delivered, the immune response will be enhanced compared to that of conventional live or killed virus vaccines. This process should translate into lower doses of live virus vaccine required to induce a robust and protective immune response. If a hundredfold lower dose is required, many more people could have access to influenza virus vaccines. This issue is clearly of paramount importance in the event of a new pandemic virus. Moreover, a live virus vaccine may give protective immunity in immunologically naive populations after a single administration, while killed virus vaccines may require high antigenic doses and a prime-boost regimen to protect against a pandemic strain. It may turn out that only live influenza virus vaccines can provide the necessary protection in case of a new pandemic. Because live influenza virus vaccines appear to be more effective in immunologically naive populations and they can be intranasally administered, they would represent a more economical way of vaccinating large numbers of people.

Replication-defective Vaccines

Other promising approaches concern the use of replication-deficient preparations. For example, virus particles that lack the gene for the nuclear export protein (NEP; formerly NS2) will go through a single cycle of replication (without forming infectious particles) (*24*). Virus particles without the M2 gene may also fit this formula (*25*). Mass production of defective viruses can be achieved by using complementing cell lines. The administration of virosomes (consisting of reconstituted viral envelopes that lack RNA), and the use of viruslike particles made by expression of viral proteins have also been shown to be effective immunization strategies against influenza (*26*,*27*). Yet another approach concerns DNA vaccination in humans by using plasmids that express $\geq$1 foreign gene. Unfortunately, this approach has been less than convincing since it appears to work best in mice and other small mammals (*28*). Thus, the jury is still out as to whether this approach is reasonable for improving influenza virus vaccines in humans.

Universal Vaccines?

Influenza viruses continue to undergo antigenic drift, which is mostly reflected in accumulating changes in the HA. This fact requires us to change the vaccine formulation or at least to reexamine the seed strains on an annual basis. Unfortunately, predicting the evolutionary change of the viral HA has not been reliable (*29*). Thus, short of

developing 20/20 foresight, predicting strain variation or the emergence of a particular pandemic strain (avian or otherwise) is unlikely (*30*). A more realistic approach is the design of more cross-protective vaccines for use in interpandemic years and during pandemics. Neirynck et al. have designed vaccines based on the conserved extracellular portion of the M2 protein fused to the hepatitis B core protein (*31*). Such an immunogen may induce a cross-reactive response in the vaccinated host. Similarly, immunization with the NA antigen is likely to induce responses that are more cross-reactive than those by the more variable HA (*32*). In both cases, however, protection will require immune responses that are more vigorous than what is seen after natural infection. Antibodies against NA and M2 proteins in infected humans are generally not protective. Thus vaccines consisting of NA or M antigens would need to be adjuvanted or otherwise made to induce a dramatically enhanced immune response. Alternatively, genetically engineered viruses could be generated, which would express several variant antigens or epitopes, thereby achieving a more cross-protective immunization. Chimeric HA recombinant viruses that express an additional 140 amino acids have recently been described (*33*). Such genetically engineered viruses may present several conserved immunogenic epitopes on the viral surface, which would be a first step toward a more universal influenza vaccine.

Conclusions

Technologies are now in place to design and construct new influenza virus vaccines that have the potential to be cheaper and more cross-protective than current vaccine preparations, while at the same time being equally safe. The greatest problems for new and better vaccines appear to be associated with regulatory hurdles and the lack of an adequate market. Regarding the bureaucratic restrictions levied on vaccines by licensing agencies, the message has to come through "that small risks have to be tolerated for larger ones to be avoided" (*34*). Also, the message needs to be disseminated to the general public that vaccines have the best cost-benefit ratio of any medical treatment and that limitations of the tort law should be considered where vaccines are concerned. The public often views vaccines and prophylactic treatments in general as being of low priority. Many people also believe they should be free. Thus, the absence of a robust commercial market is a major difficulty, resulting in slow progress for research and development of new influenza vaccines and in dangerously thin supply lines. In fact, we are far from being prepared to deal with regular influenza outbreaks, and adequate measures to cope with a pandemic outbreak are only now being considered, but are not yet in place (*35*,*36*; and http://www.washingtonpost.com/wp-dyn/content/article/2005/11/01/AR2005110101100.html).

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Mount Sinai School of Medicine holds intellectual property generated by the author in the area of reverse genetics. Dr Palese is also a consultant for several biotech and vaccine companies.

Dr Palese is a professor of microbiology and chairman of the Department of Microbiology at Mount Sinai School of Medicine. His research interests are the replication of RNA-containing viruses, especially influenza viruses, and the genetic analysis of influenza viruses to determine the functions of genes and gene products. He elucidated the genetic maps of influenza A, B, and C viruses and the precise measurements of their mutation rates and also developed the first reverse genetics technology that enabled the manipulation and analysis of influenza and other negative-strand RNA viruses. Dr. Palese is a member of the National Academy of Sciences and currently president of the American Society for Virology. He is also a senior scholar of the Ellison Medical Foundation.

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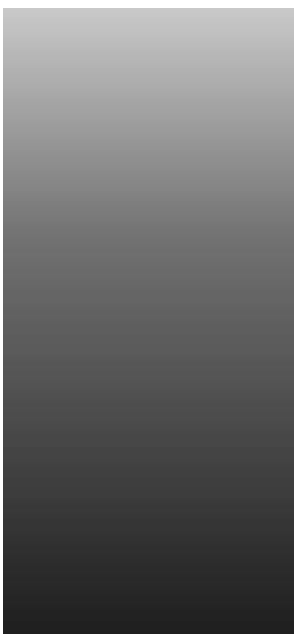
Address for correspondence: Peter Palese, 1 Gustave Levy Place, New York, NY, 10029, USA; fax: 212-722-3634; email: peter.palese@mssm.edu



Vaccines for Pandemic Influenza

Catherine J. Luke* and Kanta Subbarao*

Recent outbreaks of highly pathogenic avian influenza in Asia and associated human infections have led to a heightened level of awareness and preparation for a possible influenza pandemic. Vaccination is the best option by which spread of a pandemic virus could be prevented and severity of disease reduced. Production of live attenuated and inactivated vaccine seed viruses against avian influenza viruses, which have the potential to cause pandemics, and their testing in preclinical studies and clinical trials will establish the principles and ensure manufacturing experience that will be critical in the event of the emergence of such a virus into the human population. Studies of such vaccines will also add to our understanding of the biology of avian influenza viruses and their behavior in mammalian hosts.

Influenza is a negative-strand RNA virus that belongs to the family *Orthomyxoviridae*, which consists of 4 genera: influenza A, influenza B, influenza C, and Thogoto viruses. The proteins of influenza A are encoded on 8 RNA gene segments. Influenza A viruses are widely distributed in nature and can infect a wide variety of birds and mammals. Influenza A virus subtypes are classified on the basis of the antigenicity of their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA); 16 HA and 9 NA subtypes are known to exist, and all of them infect aquatic birds. Most infections in waterfowl are not associated with clinical disease. Relatively few subtypes of influenza A viruses have caused sustained outbreaks of disease in the human population. Influenza A viruses of the H1, H2, and H3 HA and of the N1 and N2 NA subtypes have circulated in the human population in the 20th century. H1N1 viruses appeared in 1918 and circulated until 1957, when they were replaced by H2N2 viruses. H3N2 viruses appeared in 1968, replacing H2N2 viruses, and have remained in circulation in the human population. H1N1 viruses reappeared in the human population in 1977 and continue to cocirculate with H3N2 viruses (*1*). Currently, influenza epidemics in the winter are caused by H3N2 and H1N1 influenza A and influenza B viruses.

*National Institutes of Health, Bethesda, Maryland, USA

Influenza Pandemics

In addition to seasonal influenza epidemics, influenza pandemics have occurred periodically. An influenza pandemic occurs when an influenza strain with a novel HA subtype (with or without a novel NA subtype) appears and spreads in the human population, which has little or no immunity to the novel HA. In the 20th century, pandemics occurred in 1918, 1957, and 1968 and were associated with substantial illness and death. The pandemic of 1918, the "Spanish flu," was caused by an influenza A virus of the H1N1 subtype and was responsible for ≥40 million deaths worldwide (*2*). In the Asian influenza pandemic of 1957, in which H2N2 viruses appeared, influenza-associated excess deaths were estimated at >2 million worldwide (*3*). The influenza pandemic of 1968 started in Hong Kong and was caused by an H3N2 virus. The 1968 pandemic virus had the same NA as the H2N2 virus it replaced but a novel HA. This pandemic was much less severe than the previous pandemics, with estimated influenza-associated excess deaths of ≈1 million (*3*). Preexisting immunity to the N2 NA of the 1968 pandemic influenza virus may partially explain why this pandemic was less severe than the 2 preceding pandemics, although the availability of penicillins and macrolides may also have contributed.

We cannot predict when the next influenza pandemic will occur, or which influenza virus subtype will cause it. Forecasts of the severity of the next influenza pandemic differ in their predictions of deaths based on the models used. Modeling based on the pandemic of 1968 projects 2 million–7.4 million excess deaths worldwide (*3*). Meltzer and colleagues have estimated that, in the absence of effective interventions, in the United States alone, the next influenza pandemic could cause 89,000–207,000 excess deaths and 314,000–734,000 hospitalizations, as well as tens of millions of outpatient visits and additional illnesses (*4*). In this scenario, the economic impact of an influen-

za pandemic would be severe. The economic costs due to deaths, illness, and hospitalizations in the United States alone, excluding disruptions to commerce and society, would be $71.3–$166.5 billion (*4*).

In the recent H5N1 outbreaks in Asia, >120 million birds died or were culled during a 3-month period (*3*). For the countries of Thailand and Vietnam, the estimated decreases in gross domestic product (GDP) resulting from poultry farming losses are $1.2 and $0.3 billion, respectively, with a total estimated loss in GDP for Asia of $10 to $15 billion, according to the same source. In addition to the effects on local poultry production and commerce, international trade and travel would undoubtedly suffer in an influenza pandemic.

Avian Species as a Reservoir for Pandemic Influenza Viruses

Aquatic birds are the reservoir for all known subtypes of influenza A viruses, and as such are the pool from which pandemic influenza viruses arise. Avian influenza (AI) viruses are introduced into the human population after reassortment with circulating human influenza A viruses or by directly infecting humans.

Until 1997, it was widely believed that to infect humans an AI virus would have to undergo reassortment with a human influenza virus in an intermediate mammalian species to acquire the necessary characteristics for efficient transmission to and replication in humans. In the last 10 years, direct transmission of AI viruses from birds to humans has been reported on several occasions, causing a wide spectrum of disease, ranging from mild febrile and respiratory illness in some H5 and H9N2 infections, conjunctivitis in the case of H7 influenza infections, to severe disease and death, as seen in the current H5N1 outbreak in Asia. The details of these cases are given in Table 1.

The gene segments of the influenza viruses isolated from the human H5N1 patients in 1997 were all derived from AI viruses, with no evidence of reassortment with human influenza viruses. Surveillance studies in birds in Hong Kong showed that H5N1 and H9N2 AI viruses cocirculated in poultry markets in Hong Kong at the time of the 1997 H5N1 AI outbreak, creating favorable conditions for reassortment (*16*). H9N2 AI viruses had become widespread in domestic chickens in Asia since 1990. In addition, both of these AI subtypes were isolated from ducks and geese in the region, suggesting a wide distribution in

Table 1. Direct transmission of avian influenza viruses to humans

Virus subtype	Year	Location	No. cases (no. deaths)	Clinical features	Notes	Reference(s)
H5N1	1997	Hong Kong	18 (6)		Associated with outbreak of highly pathogenic AI in poultry in the region	(*5*,*6*)
H9N2	1999	Hong Kong	2 (0)	Mild influenzalike illness		(*7*)
H9N2	1999	Guangdong Province, China	5 (0)	Mild influenzalike illness		(*8*)
H9N2	2003	Hong Kong	1 (0)	Mild influenzalike illness		(*9*)
H5N1	2003	Hong Kong	2 (1)	Primary viral pneumonia, lymphopenia, respiratory distress	7-year-old girl died in Fujian Province, China, and H5N1 infection was not confirmed. Her 33-year-old father died from confirmed H5N1 influenza infection in Hong Kong, and her 8-year-old brother recovered from H5N1 infection.	(*10*)
H7N7	2003	Netherlands	89 (1)	Conjunctivitis (78 cases), mild influenzalike symptoms (2 cases) or both (5 cases). In fatal case, pneumonia followed by respiratory distress syndrome	Most cases were in persons involved in handling poultry (86), with 3 family members also affected.	(*11*)
H10N7	2004	Egypt	2 (0)	Fever and cough	Both cases were in infants, who recovered without complications	(*12*)
H5N1	2003–present	Asia (Vietnam, Thailand, Cambodia, Indonesia)	116 (60)*	Fever, respiratory symptoms, lymphopenia, elevated liver enzymes. Severe cases progress to respiratory failure, multiple organ dysfunction, and death.	Human cases concomitant with unprecedented outbreaks of highly pathogenic H5N1 AI in poultry	WHO* (*13*–*15*)

*WHO, World Health Organization. As of September 29, 2005. Source: http://www.who.int/csr/disease/avian_influenza/country/en

avian hosts. Data from phylogenetic studies led to the hypothesis that the H5N1 Hong Kong viruses acquired their HA gene from an A/goose/Guangdong/1/96-like (H5N1) virus and the gene encoding NA from an A/teal/Hong Kong/W312/97 (H6N1)-like virus circulating in terrestrial poultry. Data also suggested that H9N2 or H6N1 AI viruses circulating in the region were the likely source of the internal protein genes (*17–20*). H9N2 viruses continue to circulate in birds in southern China.

The outbreak of human H5N1 cases in 1997 ended with the depopulation of the poultry markets in Hong Kong. These actions may have averted an influenza pandemic (*16*). Precursor viruses, however, continued to circulate in the region, and in 2003, highly pathogenic H5N1 viruses reemerged, and new human infections were identified and continue to be reported to date.

Preparing for the Next Pandemic

The reemergence of highly pathogenic H5N1 AI viruses in Asia has raised concerns of a potential pandemic, resulting in an augmented level of preparedness for such an eventuality. The pandemic preparedness plan for the United States was published in November 2005 (www.hhs.gov/pandemicflu/plan/).

Two intervention strategies could prevent or lessen the severity of an emergent influenza pandemic, vaccination and use of antiviral drugs. The use of antiviral compounds is discussed in another article in this issue (*21*). We focus on the challenges facing development of pandemic influenza vaccines and how we can prepare and test a library of vaccine seed viruses. Although the next influenza pandemic could possibly be caused by a different avian or reassortant virus than the highly pathogenic H5N1 AI virus now circulating in Asia, current vaccine development activities are largely focused on viruses of this subtype. Events in Asia underscore the urgent need for generating candidate H5N1 vaccines and evaluating them in humans, but ignoring AI viruses of the other subtypes would be imprudent. All AI viruses are presumed to have pandemic potential.

Developing Vaccines for Pandemic Influenza

Central to pandemic preparedness planning are effective vaccines to thwart the spread of a pandemic virus and to prevent illness and death associated with a novel virulent strain. The principle behind the generation of human influenza vaccines is to elicit protective antibodies directed primarily against HA, the major protective antigen of the virus that induces neutralizing antibodies. Although major advances in our understanding of the biology and ecology of the H5N1 AI viruses have been made since human infections were first reported in 1997, and we have many years of experience and much accumulated knowledge about immunity to human influenza viruses, gaps remain in our understanding of immunity to AI viruses (Table 2). Filling in these gaps is vital to developing vaccines to protect the human population. Studies using inactivated vaccines against H9N2 and H5 subtypes of AI or purified recombinant H5 HA have demonstrated that these vaccines are poorly immunogenic in comparison to epidemic human influenza strains of the H1N1 and H3N2 subtypes. For example, inactivated vaccines against avian influenza subtypes require 2 doses and administration with adjuvant to achieve the desired level of neutralizing antibody (*22–27*) (Table 3). The precise antigenic properties of a nascent pandemic strain cannot be predicted, so available vaccines may be poorly antigenically matched to the pandemic virus. Practical considerations and hurdles for pandemic influenza vaccine development also have to be overcome. Manufacturing capacity, the ability of candidate vaccine strains to grow well in eggs, and biological safety containment of parent strains for vaccine development are all problems to be addressed. In addition, the most vulnerable sections of the population may not be the same as those seen with yearly influenza epidemics, making planning to target certain population groups for vaccination difficult at best. For these reasons, the time before the next pandemic must be used judiciously for developing and clinically testing candidate vaccines.

Generating Vaccine Seed Viruses

The interpandemic period must be used to explore the optimal scientific, manufacturing, regulatory, and clinical research strategies for developing vaccines that are effective against pandemic influenza so that a vaccine will be available as soon as possible in the event of a pandemic. To this end, the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID),

Table 2. Challenges for developing vaccines for pandemic influenza: knowns and unknowns*

What we know from experience with human influenza viruses	What we don't know
Antibodies against the HA (and to a lesser extent NA) are critical for protection.	Which avian influenza virus will cross species barrier to cause a pandemic
Systemic immune response is strain specific.	Importance of antigenic drift among avian influenza viruses
Mucosal immune response provides broader cross-protection.	Immunogenicity of HA of avian viruses in humans (unknown or poor)
Cellular immunity is needed for viral clearance.	
Vaccine strain must closely match the circulating strain.	

*HA, hemagglutinin; NA, neuraminidase.

Table 3. Details of clinical trials in humans of inactivated and subunit vaccines against avian influenza

Target virus subtype	Description of vaccine candidate	Adjuvant	Findings	Reference
H9N2	Inactivated whole virus (A/HK/1073/99). 7.5, 3.8, 1.9 μg/dose with adjuvant or 15 μg without adjuvant. 2 doses, day 0 and day 21	Aluminum hydroxide	Two doses needed to achieve HI* antibody titer of ≥1:40 at any dose.	(*22*)
H9N2	H9N2 whole virus or subunit vaccine. 7.5, 15, or 30 μg per dose. 2 doses, day 0 and day 21.	None	Two doses needed to achieve HI titer of ≥1:40 in persons <32 years of age; 1 dose needed to achieve HI titer of ≥1:40 in persons >32 y of age.	(*23*)
H5N1	Low pathogenicity H5N3 strain (A/duck/Singapore/F119-3/97) subunit vaccine with or without adjuvant. 7.5, 15, 30 μg per dose. 2 doses, day 0, day 21	MF59	Geometric mean antibody and seroconversion rates significantly higher when vaccine administered with adjuvant; 2 doses of vaccine needed to achieve antibody responses indicative of protection.	(*24*)
H5N1	Purified baculovirus-expressed recombinant H5 HA derived from A/HK/156/97. 25, 45, 90 μg per dose, 2 doses or 1 dose of 90 μg followed by 10-μg dose	None	23% of volunteers had neutralizing titers of ≥1:80 after a single dose of 90 μg; 52% of volunteers had neutralizing antibody titers after 2 doses of 90 μg.	(*27*)

*HI, hemagglutination inhibition.

National Institutes of Health (NIH), is embarking on a program to develop candidate vaccines to prevent influenza pandemics caused by AI viruses. The vaccine seed viruses to be generated are based on the live attenuated cold-adapted influenza virus vaccines developed by Maassab and colleagues at the University of Michigan in the 1960s (*28*) and used as the basis for the FluMist vaccine (MedImmune, Inc., Gaithersburg, MD, USA) now licensed in the United States for persons 5–49 years of age for preventing interpandemic influenza. The principles of the development of such vaccines and safety and efficacy studies conducted in humans are reviewed elsewhere (*29*,*30*). The vaccine seed virus development strategy is not exclusive to live, attenuated vaccines, and similar studies with inactivated vaccines against different AI subtypes should be initiated.

The goal of our research program is to establish the "proof of principle" that the A/AA/6/60 cold-adapted (AA ca) virus bearing AI virus HA and NA genes will be infectious, immunogenic, and safe in humans and therefore of potential use for controlling pandemic influenza. The observed efficacy of live, attenuated vaccines for human interpandemic influenza, together with the findings to date that inactivated or subunit AI vaccines are suboptimally immunogenic in humans, strongly suggests that using live vaccines against pandemic influenza is worth exploring. Live, attenuated AI vaccines might require fewer doses and might provide broader immune responses than inactivated or subunit vaccines.

Live, attenuated influenza A candidate vaccines bearing the 6 internal genes of the AA ca donor virus (the attenuating genes) and the 2 protective HA and NA genes from human H3 or H1 viruses have been studied extensively in humans and have been licensed for general use. These vaccines are safe, infectious, immunogenic, nontransmissible, genetically stable, and efficacious (reviewed in [*30*]). It is reasonable to propose that a live, attenuated vaccine would rapidly induce protective immune responses, but this requires experimental verification in humans.

The pandemic influenza vaccine candidates will be generated by plasmid-based reverse genetics, shown in the Figure, panel A (reviewed in [*31*]). This technique allows infectious virus to be recovered from cells approved for use in human vaccine development (so-called qualified cells). These cells are cotransfected with plasmids encoding each of the 8 influenza gene segments to generate recombinant viruses that contain the HA and NA genes from AI viruses and 6 internal gene segments from the AA ca virus (*31*). Reverse genetics will allow modification of known virulence motifs in the HA or NA genes, such as the removal of the multibasic amino acid cleavage site motif in the HA gene of highly pathogenic AI strains that is associated with virulence in birds (*32*). The other advantage of reverse genetics is that a selection system is not needed to derive appropriate reassortant viruses from a background of parental viruses. In addition, the plasmids encoding the genes from the attenuated vaccine donor strain are available, and only the HA and NA genes need to be cloned for each vaccine. Several H5N1 vaccine candidates have been developed by using this technique (*33*–*36*). Some potential obstacles to applying the reverse genetics approach include the need for qualified cells for virus production and intellectual property for this technique. However, as long as the HA and NA gene segments do not have to be modified, the 6-2 gene reassortant investigational pandemic vaccines can be generated by genetic reassortment, as shown in the Figure, panel B. A candidate H9N2 pandemic vaccine was generated by using this technique (*37*).

Live, attenuated vaccines must be able to replicate to levels that elicit a protective immune response without causing disease in the host, so a balance of infectivity, level of attenuation, and immunogenicity must be

achieved. Therefore, before the next pandemic, we must evaluate in humans the safety, infectivity, immunogenicity, and phenotypic stability of live, attenuated influenza A candidate vaccines. The types of in vitro and in vivo studies that will be performed before clinical trials in humans are initiated, in addition to standard safety tests, are listed in Table 4. In vitro studies will be performed to confirm the genome sequence of the vaccine viruses. The cold-adapted and temperature-sensitive phenotype of the vaccine viruses will be confirmed in vitro in tissue culture. The attenuation phenotype of the vaccine candidates must be tested in an appropriate animal model. A critical step in evaluating vaccine candidates is selecting a model in which restriction of replication of the vaccine virus can be convincingly demonstrated in comparison to the wild-type parent virus. Since we cannot predict how AI viruses of different subtypes will behave in different animal species, animal models for each virus subtype will be developed. The use of rodent models (e.g., mice and hamsters) will be explored. The use of a ferret model will be investigated as well, although limited availability of influenza-seronegative ferrets and facilities in which highly pathogenic wild-type AI viruses can be evaluated in ferrets makes such studies logistically and practically difficult for assessing large numbers of candidate vaccines. In addition, the higher body temperature of the ferret may confound interpretation of studies in which replication of temperature-sensitive viruses is being assessed. The vaccine viruses may also require evaluation in the standard Office International des Épizooties (World Organization for Animal Health) intravenous pathogenicity test in chickens to confirm that they are not highly pathogenic in chickens and, as such, do not pose a threat to the poultry industry. Such a requirement will be guided by national agricultural authorities. Immunogenicity, dose response, antibody response kinetics, and efficacy studies will also be carried out in appropriate animal models before clinical trials.

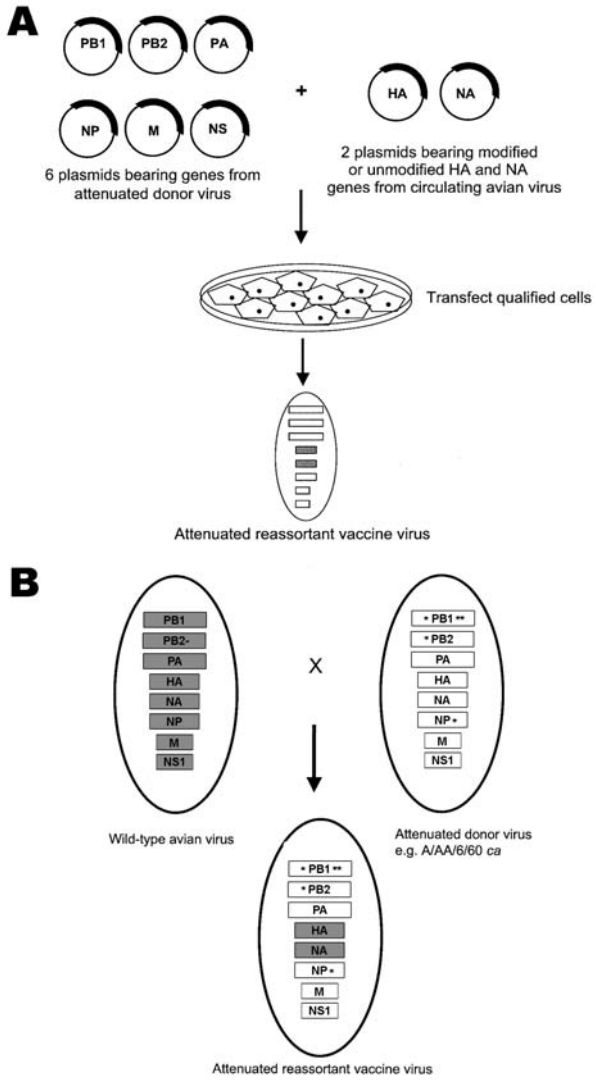


Figure. A) The 8-plasmid reverse genetics system to generate recombinant, live, attenuated pandemic influenza vaccines. Six plasmids encoding the internal genes of the attenuated donor virus are mixed with 2 plasmids encoding the circulating avian virus hemagglutinin (HA) and neuraminidase (NA) genes (which may or may not have been modified to remove virulence motifs). Qualified cells are transfected with the plasmids, and the attenuated reassortant virus is isolated. B). Generation of live, attenuated pandemic influenza vaccine viruses with the 6 internal genes from the attenuated donor virus bearing attenuating mutations (*) and the HA and NA genes from the circulating avian virus by classical reassortment. The 6-2 reassortants generated by this method are selected in the presence of antiserum specific for HA and NA of the attenuated donor virus.

Past experience with live, attenuated vaccines for interpandemic human influenza (*30*) indicates that live virus vaccines may have great potential for use as vaccines during pandemic spread of influenza because of their high level of immunogenicity for immunologically naive persons and their ability to rapidly induce immunity, i.e., within the first 10 days after vaccination. The contribution of cellular immune responses to the control of AI virus infection remains to be determined and can be examined in the context of live, attenuated vaccines. Such responses may be valuable in an influenza pandemic, in which the vaccine may protect from severe illness or death even if it is not completely antigenically matched to the emergent strain. Since a live, attenuated virus vaccine based on the AA ca donor virus has been licensed by the Food and Drug Administration for general use in healthy persons 5–49 years of age, the infrastructure for manufacture and characterization of live, attenuated virus vaccines exists. The availability of the manufacturing capability for a live, attenuated virus vaccine makes it feasible to initiate a project in collaboration with industry to develop seed viruses for live, attenuated vaccines against influenza A viruses with pandemic potential.

Table 4. Preclinical testing to be performed on live attenuated pandemic influenza vaccine candidates

In vitro testing	In vivo testing
Confirmation of virus genome sequence	Intravenous pathogenicity test in chickens
Trypsin-dependent replication in cell culture	Attenuation (restricted replication) in rodent or ferret model
Confirmation of phenotype associated with the vaccine donor virus, e.g., temperature sensitivity, cold adaptation	Immunogenicity in rodent or ferret model
	Protective efficacy in rodent model

Our overall plan includes the following steps: 1) generation of a set of live, attenuated viruses bearing an H4–H16 HA and the accompanying NA found in the wild-type virus (we will not generate novel combinations of HA and NA proteins) on the attenuated AA ca donor virus background; 2) preparation and qualification of a clinical lot of each pandemic vaccine candidate; 3) evaluation of the safety, infectivity, immunogenicity, and phenotypic stability of each candidate vaccine in humans; 4) storage of human sera obtained from vaccinees to determine antigenic relatedness of the vaccine administered to the study participant with actual newly emerged pandemic viruses; and 5) storage of seed viruses for manufacture of vaccine to prevent disease caused by pandemic viruses that do emerge. Thus, vaccine manufacture can be initiated with pretested viruses without delay. Even if the seed virus does not match the pandemic strain and a vaccine virus that is an exact match has to be generated, the dosing and immunogenicity data from the previous vaccine studies can guide its use. If the AA ca reassortant virus is safe and attenuated but infectious in humans, it can be used as a challenge virus to assess vaccine efficacy for both live and inactivated influenza virus vaccines.

A major concern associated with using a live, attenuated influenza vaccine bearing genes derived from an AI virus is the risk for reassortment of the vaccine virus with a circulating influenza virus. This reassortment could result in a novel subtype of influenza that could spread in the human population. Although such an event may not be of concern in the face of widespread disease from a pandemic strain of influenza, it would clearly be an unfavorable outcome if the threatened pandemic did not materialize. Clinical trials in humans of these live, attenuated pandemic vaccine candidates will be performed in carefully planned and executed inpatient studies. The risk for reassortment must be carefully considered by public health authorities before a decision is made to introduce a live, attenuated vaccine in a threatened pandemic. Using every available option to develop vaccines that may be used for an influenza pandemic is critical.

Conclusions

Recent events in Asia have led to intensive planning and preparation for a potential global influenza pandemic. Vaccine development is a critical part of preparedness. Recent studies that used mathematical models to study potential intervention strategies predicted that local prevaccination with a vaccine that is 70% efficacious against the pandemic strain could enhance the effectiveness of antiviral prophylaxis in preventing spread of the virus (*38*). Production and establishment of the proof of principle of candidate live and inactivated vaccines with AI HA and NA proteins in the interpandemic period could save valuable time in the event of a pandemic. Such studies will also provide information about the biology of AI viruses and immune responses to them in humans.

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Dr Luke is a regulatory officer in the Laboratory of Infectious Diseases, NIAID, NIH, where she is working on the development of vaccines against pandemic influenza.

Dr Subbarao is a senior investigator in the Laboratory of Infectious Diseases, NIAID, NIH. Her research is focused on the development of vaccines against pandemic strains of influenza and the development of animal models and evaluation of vaccines against the severe acute respiratory syndrome–associated coronavirus.

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Address for correspondence: Kanta Subbarao, Laboratory of Infectious Diseases, NIAID, NIH, Bldg 50, Rm 6132, 50, South Dr, Bethesda, MD 20892, USA; fax: 401-496-8312; email: ksubbarao@niaid.nih.gov

Pandemic Influenza Threat and Preparedness[1]

Anthony S. Fauci*

The threat of a human influenza pandemic has greatly increased over the past several years with the emergence of highly virulent avian influenza viruses, notably H5N1 viruses, which have infected humans in several Asian and European countries. Previous influenza pandemics have arrived with little or no warning, but the current widespread circulation of H5N1 viruses among avian populations and their potential for increased transmission to humans and other mammalian species may afford us an unprecedented opportunity to prepare for the next pandemic threat. The US Department of Health and Human Services is coordinating a national strategy to respond to an influenza pandemic that involves multiple agencies, including the Centers for Disease Control and Prevention, the Food and Drug Administration, and the National Institutes of Health (NIH). Within NIH, the National Institute of Allergy and Infectious Diseases (NIAID) conducts basic and clinical research to develop new vaccine technologies and antiviral drugs against influenza viruses. We describe recent research progress in preparing for pandemic influenza.

Since December 2003, H5N1 avian influenza viruses have killed millions of domestic fowl in Southeast Asia (tens of millions more have been culled). It has also infected >130 persons and killed >70 in Vietnam, Thailand, Cambodia, Indonesia, and China (Figure 1) (*1*). If the virus acquires the ability to transmit readily among humans, an influenza pandemic could ensue, with the potential to kill millions of people (*2*). Reports in both the popular press (*3*) and scientific literature (*4–7*) have raised alarms in the United States and throughout the world. The prospect of pandemic influenza provides good reason to be concerned. Rather than react in panic, however, we need to determine what can be done now with the knowledge and resources currently available to prevent or minimize the impact of a potential pandemic. At the same time we must ask how we can improve our infrastructure and technology to prepare for future outbreaks.

Unlike seasonal influenza epidemics caused by viruses that mutate in small but important ways from year to year, a process known as antigenic drift, pandemic influenza is caused by a virus that is dramatically different from those that have circulated previously, which can occur through a phenomenon referred to as antigenic shift (*2*). Such viruses can cause pandemics because few people, or none at all, have had prior immunologic exposure to surface proteins of these viruses. In a typical interpandemic influenza season, people may have some residual immunity from exposure to previously circulating influenza strains or from vaccinations (*8*). For example, the predominant circulating influenza virus in the Northern Hemisphere during the 2004–2005 influenza season was an H3N2 virus that had drifted somewhat but was still fundamentally similar to the H3N2 viruses that had circulated in 2003–2004 and previously. Nonetheless, a virus that has undergone antigenic drift can cause illness and death; vaccination provides varying degrees of protection from severe illness and death from influenza complications (*8*). Pandemic influenza, however, can cause a public health crisis because most people would be immunologically naive to the new virus. In addition, the pandemic virus might be inherently more virulent than interpandemic strains. Whereas seasonal influenza rarely threatens the lives of young and otherwise healthy persons, pandemic influenza frequently has exacted a serious toll in healthy, young adults (*2,9*).

As of December 2005, outbreaks of H5N1 avian influenza viruses had occurred in domestic poultry populations in at least 16 countries in Asia and eastern Europe

*National Institutes of Health, Bethesda, Maryland, USA

[1]This article is dedicated to the memory of John La Montagne who died suddenly and unexpectedly in November 2004. Dr. La Montagne was the deputy director of NIAID and a world leader in the fields of influenza and emerging infectious diseases. He is sorely missed by his many colleagues.

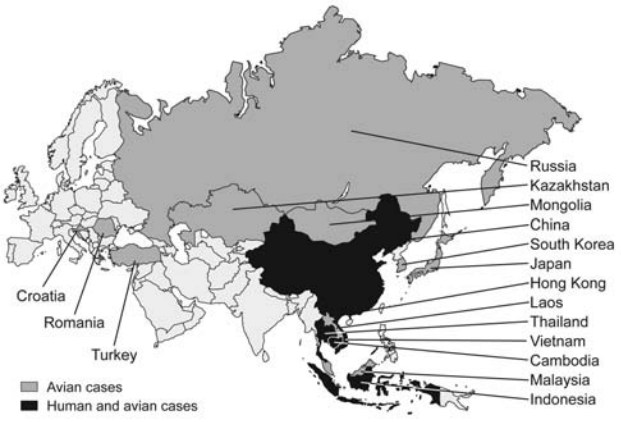

Figure 1. H5N1 cases in Asia, 2004–2005, among birds (dark gray) and humans (black) (*1*). A total of 137 laboratory-confirmed cases, including 70 deaths, occurred. This total includes 22 human cases and 14 deaths in Thailand, 93 human cases and 42 deaths in Vietnam, 4 human cases and 4 deaths in Cambodia, 13 human cases and 8 deaths in Indonesia, and 5 human cases and 2 deaths in China.

(*10*). H5N1 viruses have also have been isolated from wild birds. Disease caused by H5N1 and presence of the virus among thousands of migratory wildfowl have been observed in western China, and more recently, in Kazakhstan, Mongolia, and Croatia, which raises the possibility that H5N1 may be spreading from its stronghold in Southeast Asia through migratory flyways (*11–13*). In addition to a growing list of avian species, the virus has infected several mammalian species, including tigers, leopards, and pigs, and transmission among domestic cats has been observed in the laboratory (*14–16*). Together, these findings suggest that both the geographic and host ranges of H5N1 viruses are expanding.

The true extent of human H5N1 infections is not precisely known; preliminary reports suggest that the extent of bird-to-human transmission may be more widespread than originally thought (*17*). Thus far, the virus has not acquired the ability to be efficiently transmitted from human to human, although a recent report describes the possible transmission of H5N1 within a family in Thailand (*18*).

The H5N1 avian influenza viruses now circulating may be the most likely candidates for triggering an influenza pandemic because of ongoing reports of new cases in humans (*19*). However, other avian influenza viruses also are being monitored for their potential to infect and cause disease in humans (Figure 2). The H9N2 influenza virus, although not highly pathogenic, has circulated widely among birds in Hong Kong and China; it infected 2 children in 1999 (*20*,*21*) and 1 child in Hong Kong in 2003 (*22*), each of whom recovered. Five additional human infections with H9N2 viruses were reported in the Chinese literature (*23*). Another avian influenza virus, H7N7, is worrisome because it is highly pathogenic in birds and appears to be more readily transmissible from human to human (*24*,*25*). During a large outbreak of highly pathogenic avian influenza in Europe in 2003, an H7N7 virus was detected in at least 86 poultry workers and 3 family members who had no contact with chickens; these persons were treated for conjunctivitis, influenzalike symptoms, or both. A veterinarian who handled infected chickens died of pneumonia and acute respiratory distress (*24*,*25*). With the exception of this fatal case, the H7N7 virus appeared to be relatively benign for humans. Recent reports indicate that an H7 influenza A virus may be circulating among chickens in North Korea (*26*). If a virus such as H5N1 (which is highly pathogenic in humans) were to acquire the genetic capability that enabled the efficient transmissibility observed with H7N7 or human H1N1 or H3N2 influenza viruses, while maintaining most or all of its pathogenic potential, a deadly pandemic could ensue.

Predicting or preventing the natural events that could facilitate efficient transmission of a pandemic influenza virus among humans is difficult. However, we must be prepared to react quickly and decisively should such an event occur. Critical to the containment of a potential influenza pandemic is diligent surveillance for novel viruses in both human and animal populations using appropriate diagnostics; we must also monitor the viruses for changes that could signal increased virulence or transmissibility. Equally important are the development and production of effective countermeasures, such as vaccines and antiviral drugs (*27*).

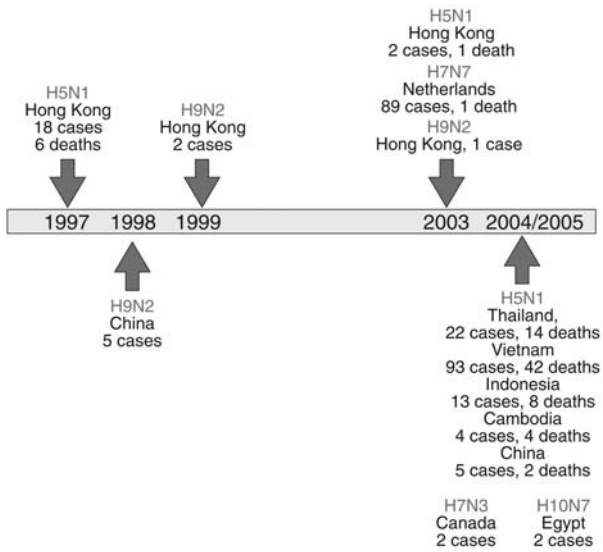

Figure 2. Timeline of documented human infection with avian influenza viruses, 1997–present (*2*). Sporadic cases of mild human disease associated with avian influenza viruses were reported before 1997.

Pandemic Influenza Preparedness

Vaccine development is a critical component of pandemic influenza preparedness. In this regard, the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH) in April 2005 initiated a phase I clinical trial to assess the safety and immunogenicity of different doses of an inactivated H5N1 influenza vaccine manufactured by Sanofi Pasteur (*28*). In this study, 451 healthy adult volunteers were vaccinated at 3 sites in the United States. Preliminary evaluation indicates the vaccine is safe and able to stimulate an immune response that may be protective. The vaccine is currently being tested in the elderly, and testing in children is expected to begin by January 2006.

The H5N1 seed virus used to make this vaccine was developed in a matter of weeks through the use of reverse genetics, whereas the traditional process of genetic reassortment usually requires a longer period of time and is less predictable (*29*). Additional pilot lots of inactivated vaccine are being produced by another manufacturer, Chiron Corporation, and are expected to undergo testing by early 2006.

Chiron also has produced 40,000 doses of an inactivated H9N2 influenza vaccine formulated with and without MF59 adjuvant. Clinical trials to test the safety and immunogenicity of the inactivated H9N2 vaccine are underway, with promising preliminary results. In addition, the US Department of Health and Human Services (HHS) has awarded several contracts to pharmaceutical companies to produce large quantities of bulk H5N1 vaccine as part of the HHS Pandemic Influenza Preparedness Program. These contracts are a critical step toward pandemic influenza preparedness because they pave the way for the manufacturer to commence efficient, large-scale production of any pandemic vaccine if or when it is needed. HHS has also awarded a separate contract to Sanofi Pasteur to accelerate the development of cell culture–based technologies for influenza vaccine production (*28*).

In addition, the intramural research program of NIAID has generated live, attenuated, cold-adapted H9N2 and H5N1 vaccine candidates that have proven protective in mice. The H9N2 vaccine candidate has been tested in a phase I clinical trial, and data are currently being evaluated; clinical evaluation of the H5N1 vaccine is planned for the spring of 2006. Live, attenuated vaccines are especially promising because they generally trigger more rapid and robust immune responses compared with those induced by inactivated vaccines. Live, attenuated vaccines may also offer more cross-reactivity and therefore greater protection against different variants of the same virus (*30*).

The concept of extending vaccine supplies also is being pursued. Research has suggested that delivering vaccines intradermally might allow successful immunization with less antigen (*31–33*); clinical trials to compare intramuscular versus intradermal delivery of H5N1 vaccines began in 2005. Preliminary safety data showed no adverse effects and immunogenicity data are expected soon. Studies to assess the effect of alum and MF59 adjuvants on inactivated H5N1 vaccine safety and immunogenicity also are anticipated.

Other research efforts are focused on medications to treat influenza infection. Unfortunately, most currently circulating H5N1 influenza viruses are resistant to 2 inexpensive antiinfluenza drugs, rimantadine and amantadine, that target the viral M2 protein. Newer drugs such as oseltamivir phosphate and zanamivir that target the influenza neuraminidase protein appear to be effective against most current H5N1 strains (*34*). HHS and the Centers for Disease Control and Prevention have begun developing a stockpile of antiinfluenza drugs that includes oseltamivir phosphate, zanamivir, and rimantadine for future use should pandemic influenza occur. Numerous other projects are under way to identify novel drug targets and develop compounds that inhibit viral entry, replication, and maturation (*28*).

Underpinning these efforts are basic research studies. For example, NIAID coordinates the Influenza Genome Sequencing Project, a collaborative effort to create complete genetic blueprints of known human and avian influenza viruses. As of December 7, 2005, a total of 559 influenza genome sequences have been made publicly available in GenBank by the NIAID project (*35*). In a separate but related contract awarded to researchers at St. Jude Children's Research Hospital, animal influenza viruses from wild birds, live bird markets, and pigs in Hong Kong and North America are being sequenced, and surveillance has expanded to include additional sites in Asia. The goal of these projects is to rapidly sequence influenza genomes derived from a variety of human and animal sources to enable scientists to understand how the viruses evolve, spread, and cause disease. The long-term goal is improving methods of prevention and treatment.

The Fragile Vaccine Enterprise

As we develop strategies to prepare for an influenza pandemic, we need to address the overall fragility of the entire vaccine research and manufacturing enterprise (*27*,*36*,*37*). Many pharmaceutical companies are reluctant to enter or remain in the business of manufacturing vaccines. Unpredictable consumer demands and lack of financial incentives make vaccine manufacturing a risky business in today's marketplace. This situation is particularly true with influenza vaccine. Strong collaborations among government, academia, and industry are needed to ensure a reliable vaccine supply. The biomedical research community can help by developing state-of-the-art

technologies and sharing them with industry to streamline the manufacturing process and make it more flexible, predictable, and able to adapt to the evolving nature of influenza viruses and other pathogens. Financial and economic incentives, including fair pricing and guaranteed purchase of unsold supplies, regulatory relief, tax incentives, liability protection, and intellectual property considerations, are needed to ensure a steady supply of vaccines (*27*,*36*,*37*). Although the fragility of the vaccine industry cannot be fixed overnight, the process needs to be initiated now to adequately prepare for future pandemics.

Lessons from Severe Acute Respiratory Syndrome

Recent experience with an outbreak of severe acute respiratory syndrome (SARS) serves as an instructive example in preparing for a potential influenza pandemic (*38*,*39*). In 2002, the deadly respiratory disease emerged and rapidly spread to Canada, Vietnam, Hong Kong, and other sites in China, ultimately resulting in 8,098 cases and 774 deaths. The outbreak, which elicited a classic study in epidemiologic investigation with regard to identifying the point source, tracking the spread, and instituting containment measures, taught us many important lessons. Academic scientists, public health officials, and commercial pharmaceutical companies acted together in an unprecedented way, leading to the development of promising vaccine candidates in record time. The etiologic agent of SARS, a previously unrecognized coronavirus, was identified in March 2003 and sequenced within 2 weeks, and a vaccine candidate was developed by the following March. In December 2004, a clinical trial of a candidate SARS vaccine began at the NIH Vaccine Research Center (*40*).

Because the SARS coronavirus is not as easily transmitted as influenza viruses, we do not know whether the actions that led to the containment of SARS would be as successful if an avian influenza virus acquired the ability to spread efficiently from person to person. However, we have an added advantage in bracing for pandemic influenza that we did not have with SARS. As noted, SARS is caused by a coronavirus that was unknown before the 2003 outbreak. In the current situation, we have identified the H5N1 virus as a likely candidate for triggering a pandemic.

We cannot be certain when the next influenza pandemic will emerge, or even whether it will be caused by H5N1 or an unrelated virus. However, we can be certain that an influenza pandemic eventually will occur. The efforts currently under way to monitor the evolution and spread of H5N1 and other influenza viruses and to develop candidate vaccines and appropriate countermeasures will help in developing the infrastructure and manufacturing capacity that will be required to scale up vaccine and antiviral production when the pandemic occurs.

Because quantities of vaccine and antiviral drugs against a pandemic influenza virus will be limited, deciding beforehand how to best use our resources throughout the world to minimize the impact of pandemic influenza is critical. Global cooperation will be vital. During the SARS epidemic, the World Health Organization created an outstanding network of laboratories and public health agencies from countries around the globe that were indispensable in identifying and ultimately containing the spread of the virus. To adequately address the many research issues surrounding avian influenza and other potential pandemic pathogens, NIAID's Office of Clinical Research is establishing a Southeast Asia Clinical Trials Network to evaluate influenza interventions. This network builds upon existing infrastructure where possible and will be a true partnership between the investigators and the healthcare leadership of the target countries. Such international teamwork is essential as we prepare for an influenza pandemic.

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Dr Fauci is director of NIAID of the National Institutes of Health in Bethesda, Maryland. He oversees a portfolio of basic and applied research to prevent, diagnose, and treat infectious diseases, including influenza.

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Address for correspondence: Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg 31, Room 7A03, MSC 2520, Bethesda, MD 20892-2520, USA; fax: 301-496-4409; email: afauci@niaid.nih.gov

Dead Bird, by the influential 19th-century American artist Albert Pinkham Ryder (1847–1917), was first seen by Duncan Phillips no later than 1916 but was not purchased for the collection until it became available a decade later. The major scholarly catalog of The Phillips Collection, The Eye of Duncan Phillips: a Collection in the Making (*1*), calls Dead Bird "one of Ryder's most powerful images," noting that it "explores a recurrent illusory theme: the coexistence of the corporeal and the ethereal," and that "[s]uch starkly realistic details as the rigidly curled claws, rendered in heavy impasto, and the subtle textured contrasts of plumage and beak, create a moving evocation of suffering and death." The Phillips Collection, Washington, DC. Reproduced with permission.

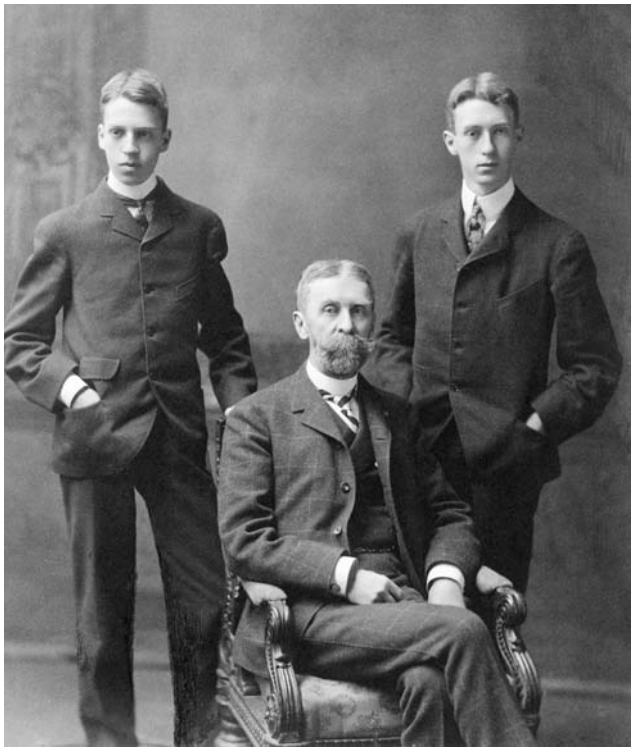
Influenza and the Origins of The Phillips Collection, Washington, DC

David M. Morens* and Jeffery K. Taubenberger†

The two Phillips brothers were so inseparable that when James, the older, was ready to leave home for Yale in 1902, he waited 2 years so that Duncan, the younger, could graduate from secondary school and accompany him. The brothers, who were full of energy and talent, spent their early years in Pittsburgh, where their maternal grandfather, James Laughlin Phillips, had achieved success as a banker and cofounder of the Jones and Laughlin Steel Company. Seeking a milder climate because of his health, the boys' father, Major Duncan Clinch Phillips, relocated the family to Washington, DC. In college, Duncan (the son) was elected an editor of the Yale Literary Magazine. Soon after college, James was appointed assistant treasurer of the Republican Party. Both developed a passionate love of contemporary art, and in 1916 their efforts to identify and purchase modern paintings had become so successful that James requested an annual stipend of $10,000 from their parents for the purchase of works of art for their growing collection.

But war had already broken out in Europe, and in 1917 the United States entered it. The brothers' patriotism overtook them, and they tried to enlist, even though they were pacifists at heart. Both were rejected for service. Duncan, turned down by both the Army and the Navy, was 30–40 pounds under the desired weight for his height, which suggested to recruiters the possibility of a chronic disease he in fact did not have. James had had prior bouts of pneumonia, and his military rejection may have been related to questions about his pulmonary status. Disappointed, James

*National Institutes of Health, Bethesda, Maryland, USA; and †Armed Forces Institute of Pathology, Washington, DC, USA



Duncan Clinch Phillips, Jr (left), his father Major Duncan Clinch Phillips (seated), and his brother James Laughlin Phillips, who died of influenza in October 1918. Photograph used with permission of The Phillips Collection, Washington, DC.

nonetheless arranged to marry his sweetheart Alice, with Duncan as best man.

But as was the case for so many in those dark years, the world was beginning to unravel. Their father died suddenly not long after the wedding. Surrounded by war and loss, James and Alice moved to Chevy Chase, Maryland, near the headquarters of the American Red Cross, where James became associate director of the Bureau of Personnel, in charge of applications for overseas war service. Then, in the fall of 1918, the "Spanish flu" struck James, and on October 21, he died in the family home in nearby Washington, DC. Her son's death broke the health of their mother, who became a semi-invalid. His secure world shattered, Duncan's health broke down too, and he gave in to despair.

"There came a time when sorrow all but overwhelmed me," he later wrote. "Then I turned to my love of painting for the will to live. Art offers two great gifts of emotion—the emotion of recognition and the emotion of escape. Both emotions take us out of the boundaries of self…. So in 1918 I incorporated the Phillips Memorial Gallery… to create a Memorial worthy of… my father… and my brother, James Laughlin Phillips, an idealist… a keen student of men and social conditions—a broad-minded, warm-hearted, lovable and very noble American" (*2*).

And so as a direct consequence of the death of his brother James from influenza, the 32-year-old Duncan Clinch Phillips, Jr (1886–1966) dedicated his life to creating a living memorial to him and to their father, and to establishing one of the finest public museums of modern art in the world. The collection, assembled over the next 5 decades, showed his remarkable taste, vision, and prescience in recognizing great works before others had suspected their greatness. Duncan's creative expression of feeling, the product of an artistic spirit, is reminiscent of similar creative expressions in literary form: the beautiful stories of Thomas Wolfe and Katherine Anne Porter, both of whom wrote about death and suffering from influenza. Wolfe's remarkable scene in Look Homeward, Angel (*3*) records the death of his own brother Benjamin from Spanish influenza, 2 days before the death of James Phillips. In Pale Horse, Pale Rider (*4*), Porter wrote a surrealistic but harrowing account of her own near death from influenza in 1918 and her belated discovery of the death from influenza of the lover who had cared for her. In each case, unbearable tragedy and loss were ennobled by art.

The collection assembled by Duncan Phillips and his wife Marjorie, herself a painter, focuses on modern art and its sources. The nearly 2,500 items include works by many now-famous 19th and 20th-century artists (van Gogh, Degas, Homer, Kandinsky, Klee, Matisse, O'Keeffe, Rothko) as well as earlier artists whose work Phillips believed anticipated modern art (Chardin, Goya, El Greco, Daumier). Phillips also championed many artists who were not well known at the time (Milton Avery, Pierre Bonnard, Karl Knaths, John Graham, Nicolas de Staël) and sometimes provided stipends to them (Arthur Dove, Augustus Vincent Tack).

Today The Phillips Collection is still housed in the family home, where James died, at 21st and Q Street, in northwest Washington, DC. The building itself is a work of architectural accomplishment, built in Georgian Revival style by Hornblower and Marshall in 1897. The paintings are exhibited in a warm intimate setting that encourages reflection and contemplation. Even though The Phillips Collection was conceived in sorrow and loss, Duncan Phillips wanted the viewing experience to be "joy-giving and life-enhancing" (*1*).

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Address for correspondence: David M. Morens, National Institutes of Health, 6610 Rockledge Dr, Room 4097, Bethesda, MD, 20892 USA; fax: 301-480-1594; email: dm270q@nih.gov

Nonpharmaceutical Interventions for Pandemic Influenza, International Measures

World Health Organization Writing Group*[1]

Since global availability of vaccine and antiviral agents against influenza caused by novel human subtypes is insufficient, the World Health Organization (WHO) recommends nonpharmaceutical public health interventions to contain infection, delay spread, and reduce the impact of pandemic disease. Virus transmission characteristics will not be completely known in advance, but difficulties in influenza control typically include peak infectivity early in illness, a short interval between cases, and to a lesser extent, transmission from persons with incubating or asymptomatic infection. Screening and quarantining entering travelers at international borders did not substantially delay virus introduction in past pandemics, except in some island countries, and will likely be even less effective in the modern era. Instead, WHO recommends providing information to international travelers and possibly screening travelers departing countries with transmissible human infection. The principal focus of interventions against pandemic influenza spread should be at national and community levels rather than international borders.

Pandemic preparedness ideally would include pharmaceutical countermeasures (vaccine and antiviral drugs), but for the foreseeable future, such measures will not be available for the global population of >6 billion (*1*). Thus, in 2005, after consultations with experts, the World Health Organization (WHO) recommended nonpharmaceutical public health interventions in its updated global influenza preparedness plan (*2*). The recommendations are intended as guidance, not as formal WHO advice (*3*). Such interventions, designed to reduce exposure of susceptible persons to an infectious agent, were commonly used for infection control in previous centuries. This report (part 1) and a companion article (part 2 [*4*]) summarize the scientific data, historic experience, and contemporary observations that make up the limited evidence base for these interventions as applied to influenza. Part 1 summarizes the relevant transmission characteristics of influenza and the basis for interventions to prevent spread from 1 country to another; part 2 summarizes the basis for measures within countries at the national and community levels. Both parts are designed to be read in conjunction with WHO recommendations (*2*,*3*).

Nonpharmaceutical interventions outside of healthcare settings focus on measures to 1) limit international spread of the virus (e.g., travel screening and restrictions); 2) reduce spread within national and local populations (e.g., isolation and treatment of ill persons; monitoring and possible quarantine of exposed persons; and social distancing measures, such as cancellation of mass gatherings and closure of schools); 3) reduce an individual person's risk for infection (e.g., hand hygiene); and 4) communicate risk to the public. We discuss the first category; categories 2 and 3 are addressed in part 2. We do not address infection control measures for patient care or risk communication.

*World Health Organization, Geneva, Switzerland

[1]The writing group was established by request of the WHO Global Influenza Programme. It consisted of the following persons: David Bell, Centers for Disease Control and Prevention, Atlanta, Georgia, USA (coordinator); Angus Nicoll, European Centre for Disease Prevention and Control, Stockholm, Sweden, and Health Protection Agency, London, United Kingdom (working group chair); Keiji Fukuda, WHO, Geneva, Switzerland; Peter Horby, WHO, Hanoi, Vietnam; and Arnold Monto, University of Michigan, Ann Arbor, Michigan, USA. In addition, the following persons made substantial contributions: Frederick Hayden, University of Virginia, Charlottesville, Virginia, USA; Clare Wylks and Lance Sanders, Australian Government Department of Health and Ageing, Canberra, Australian Capital Territory, Australia; and Jonathan Van Tam, Health Protection Agency, London, United Kingdom.

Transmission Characteristics of Influenza Viruses

Most information on transmission of influenza viruses is based on older experimental studies, inference from observations during outbreaks, and studies with other objectives, especially the assessment of vaccine or drug efficacy. These sources have substantial limitations: investigations often used different methods, involved small numbers of persons, and reflected the behavior of influenza A and B viruses in seasonal rather than pandemic settings (the level of preexisting immunity in populations is substantially higher in seasonal epidemics). For this reason, data from young children, who presumably lack prior exposure and therefore immunity to influenza, may better reflect illness and viral shedding patterns of pandemic disease. The "infectiousness" of patients is virtually always inferred on the basis of viral shedding from the upper respiratory tract rather than from directly observed transmission, but the relationship between nasopharyngeal shedding and transmission is uncertain and could vary. Detailed studies of lower respiratory tract virus loads, particularly relevant to small-particle aerosol transmission during coughing and sneezing, are not available. In many studies, the preexisting influenza antibody status of study participants is not reported, even though this factor is critical in influencing illness and viral shedding patterns. In controlled studies, in which susceptible study participants are typically screened for preexisting influenza antibody by hemagglutination inhibition assays to the challenge virus, the routes of infection and the challenge virus can differ. Other factors that differ among studies are the age and preexisting medical conditions of study participants and the timing of specimen collections for virus testing.

Viral Shedding and Transmission by Persons with Symptoms

In otherwise healthy adults with influenza infection, viral shedding 24–48 h before illness onset has been detected but generally at much lower titers than during the symptomatic period (for more details see Appendix, available online from http://www.cdc.gov/ncidod/EID/vol12no01/05-1370_app.htm). Titers of infectious virus peak during the first 24–72 h of illness (10^3–10^7 50% tissue culture infective dose [$TCID_{50}$]/mL nasopharyngeal wash) and decline within several days, with titers usually low or undetectable by day 5. Shedding in highly immunocompromised persons may last weeks to months. Compared with adults, children can shed virus earlier before illness begins and for longer periods once illness starts. As in adults, peak shedding in children occurs during the first 1–3 days of illness, but absolute levels may be higher than those in adults. In 1 report, at least 4 illnesses (8% of the total) in children were associated with presymptomatic shedding that began 6, 4, 3, and 3 days, respectively, before illness onset (*5*). The median duration of virus detection is typically 7–8 days after illness onset, but shedding for up to 21 days has been recorded. In 1 study, virus was shed by 10% of children on days 8–11, by 5% on days 12–15, and by 0% on days 16–19 (*6*). Infants with infection requiring hospitalization may shed virus longer. In both adults and children, shedding does not usually continue once illness has resolved. Serologic testing indicates that ≈30%–50% of seasonal influenza infections may not result in illness.

Viral Shedding and Transmission by Infected Persons without Symptoms

During the incubation period, persons with presymptomatic influenza infection shed virus at lower titers than persons with symptoms (online Appendix); however, the infectiousness of those with presymptomatic infection has not been studied. Apparently the only published report implicating transmission during the incubation period involves a group of adults in New Zealand in 1991. Of 26 adults who bagged fertilizer for 8 h, influenzalike illness (fever, headache, sore throat, myalgia, respiratory symptoms) developed in 16 and mild, "cold-like" illnesses developed in 3 persons within 24 to 48 h after working with the fertilizer. A person considered to be the probable index patient had felt unwell during work, although he did not have respiratory symptoms; an influenzalike illness began to develop 6 h after he finished work. Influenza A virus H1N1 was isolated from 2 symptomatic persons; whether these included the suspected index patient and whether that person transmitted infection during an incubation period or the cluster resulted from community exposure are unknown. The group shared drinking bottles and worked in a dusty environment, both of which could have facilitated transmission (*7*).

Large-Droplet and Aerosol Respiratory Transmission

Animal studies and most influenza outbreaks among humans suggest that virus-laden large droplets (particles >5 μm in diameter) generated when infected persons cough or sneeze are the predominant mechanism of influenza virus transmission (*8*). However, evidence for aerosol spread (especially in unventilated conditions) is available (*9*). Although a direct comparison has not been made, experimental studies suggest that the infectious dose for humans exposed by aerosol is lower than that seen with experimental nasopharyngeal instillation (*10*). The precise proportion of infections transmitted by large droplets versus aerosols is difficult to assess and likely depends on the setting but is relevant when developing recommendations on mask use. Data do not exist to quantify the relative efficacy of surgical masks versus respirators in preventing

influenza infections in exposed persons, but surgical masks should protect against large droplets, believed to be the major mode of transmission (*8*).

Transmission by Contaminated Hands, Other Surfaces, or Fomites

Transmission of influenza viruses by contaminated hands, other surfaces, or fomites has not been extensively documented but is believed to occur. In a nursing home outbreak in Hawaii, an investigation concluded that transmission of oral secretions from patient to patient by staff who were not gloved best explained the outbreak (*11*). In an environmental survival study, influenza A virus placed on hard, nonporous surfaces (steel and plastic) could be cultured from the surfaces at diminishing titer for <24 to 48 h and from cloth, paper, and tissues for <8 to 12 h at conditions of 35% to 40% humidity and a temperature of 28°C (*12*). Higher humidity shortened virus survival. Virus on nonporous surfaces could be transferred to hands 24 h after the surface was contaminated, while tissues could transfer virus to hands for 15 min after the tissue was contaminated. On hands, virus concentration fell by 100- to 1,000-fold within 5 min after transfer. The authors concluded that transmitting infection from the surfaces tested would require a high titer of virus ($10^{5.0}$ $TCID_{50}$/mL) on the surface; such titers can be found in nasal secretions at an early stage of illness.

Incubation Period and Infectiousness

The incubation period for influenza averages 2 days (range 1–4 days), and the serial interval (the mean interval between onset of illness in 2 successive patients in a chain of transmission) is 2–4 days. Also, viral excretion peaks early in illness. These factors enable influenza to spread rapidly through communities. By contrast, severe acute respiratory syndrome (SARS) has a serial interval of 8 to 10 days, and peak infectivity does not occur until week 2 of illness, which allows more time to effectively implement isolation and quarantine measures (*13*). The basic reproduction number (R_0, the mean number of secondary cases generated by 1 infected person in a fully susceptible population) of the 1918 pandemic influenza subtype has recently been re-estimated as ≈2–3 (*14*) and 1.8 (*15*), comparable to that of the SARS-associated coronavirus (SARS-CoV) (R_0 2–4) (*13*).

Amplifying Groups and Settings

Children in preschool and school-age groups are frequently observed to amplify transmission (*16*), although any group living in close proximity can do so, and outbreaks are observed in institutions involving persons of all ages (*11*). Although transmission may be amplified at mass gatherings (e.g., theaters, sports events), documentation is scarce.

Slowing or Preventing International Spread of Pandemic Influenza

Experience from Earlier Pandemics

1918 Experience with Quarantine Enacted by Islands

In the 1918 pandemic, some island countries enacted maritime quarantines that appear to have delayed or prevented the introduction of pandemic influenza. Maritime quarantines were facilitated because ships had often been at sea for an extended period, reducing the likelihood of ongoing onboard infection at the time of arrival in port. Also, authorities could require ships to anchor in harbors or at quarantine stations on offshore islands, thus minimizing contact with persons on shore.

In October 1918, Australia began to quarantine arriving ships upon which a case of influenza had occurred during the voyage; the duration of quarantine was determined on the basis of the date of the most recent case. Quarantine was also applied for 7 days, even if no cases were reported, to vessels arriving from New Zealand and South Africa because of severe epidemic disease in those areas and from certain Pacific Islands with which communication was limited. Persons in quarantine had their temperature measured at least once daily, and those with an oral temperature ≥99°F (37.2°C) were isolated at hospitals for observation. Measures taken by hospital staff to avoid infection included the use of masks and other "routine precautions taken at isolation hospitals." Reportedly, no direct evidence of escape of infection from any vessel to the shore occurred.

From October 1918 through May 1919, a total of 79 "infected vessels" containing 2,795 patients, 48,072 passengers, and 10,456 crew and 149 "uninfected vessels" containing 7,075 passengers and 7,941 crew arrived at Australian ports (*17*,*18*). The first cases of pandemic influenza in Australia were reported in January 1919, suggesting that these measures delayed entry of the disease for ≈3 months. Although the national quarantine director believed that pandemic influenza had entered Australia before quarantine was established, this belief was not documented, and other reports indicate that some ships' officers and soldiers returning to Australia from Europe had concealed illness to avoid protracted quarantine (*18*). When the infection did emerge in Australia, case-fatality rates were lower than those in many places affected earlier.

According to a report from the New South Wales Department of Public Health, ships with ill passengers arrived regularly at Sydney (the state capital) from October 1918 to January 1919. Of 326 passengers or crew treated at the quarantine hospital, 49 died. Recovered patients and contacts emerging from quarantine were released into the general population and monitored by health officials for a

few days to a few weeks. Two cases were in nurses who had contracted influenza while caring for patients at the quarantine hospital. "In no case did any suspicion arise that such persons had spread influenza among those with whom they had come in contact" (*19*). The first cases of influenza in New South Wales were in soldiers who arrived overland by train from the port city of Melbourne, Victoria, where recent cases were known to have occurred but were not promptly disclosed by the authorities (*19*).

In 1918, the island of Madagascar, then a French colony, also implemented a "rigorous quarantine" and did not report cases of influenza until April 1919. In contrast, nearby coastal regions of eastern and southern Africa reported cases beginning in September to December 1918. Contact between Madagascar and South Africa, where the disease was epidemic, was limited to a single coastal steamboat (*20*,*21*). In the Pacific, American Samoa implemented quarantine measures and was spared infection, while nearby islands were severely affected (*22*). The French colony of New Caledonia was spared infection by requiring ships to remain in quarantine at their ports of departure, a form of "exit screening," discussed below (*23*).

Other Quarantine Experiences

On the African mainland, quarantine was enacted in 1918 in some port cities in, for example, Liberia, Gabon, and Ghana (formerly known as the Gold Coast). Details generally are unavailable, but, on the whole, even though entry may have been delayed by some weeks, the experience was less successful than that of islands that enacted quarantine. Disease arrived from inland routes and, according to 1 report, quarantine of a ship in Accra, Ghana, known in advance to be carrying persons with influenza was not successful; disease spread to dock workers and subsequently entered the country (*21*,*24*).

In 1918, closing roads at the northern land border of Ghana was not feasible because of the volume of trade and the probability that police barriers would be evaded. An attempt was nevertheless made to close roads at the border town of Tumu, but authorities concluded that "a handful of constables could not stop the epidemic and the effort was soon abandoned" (*24*). In Canada and Australia, substantial measures, including police checkpoints and interruption of road and rail traffic, did not prevent or appear to delay the spread of infection between Canadian provinces or Australian states (*4*,*18*).

A WHO expert consultation on the 1957 influenza pandemic summarized the effect of quarantine measures at international borders as follows. Onset in Israel was delayed by 2 months in comparison to neighboring countries, attributed to absence of international travel with neighboring countries (for political, not quarantine reasons). In South Africa, "some delay" occurred from restrictions on ships arriving at ports, but the evidence was "less convincing." Elsewhere, "no effect was detected. It seems that if such measures are to be effective, they must be very severe…. a high price to pay for a few additional weeks freedom from the disease" (*25*).

Experience from Contemporary SARS and Influenza Outbreaks

In modern times, the most extensive use of nonpharmaceutical public health interventions to contain a transmissible respiratory viral infection occurred during the SARS epidemic of 2003. Some lessons learned from that experience may be applicable to influenza, although important differences exist between the epidemiologic parameters of influenza virus and SARS-CoV. The most notable of these are that influenza has a serial interval of 2 to 4 days and infectivity is maximal early in illness, whereas for SARS the serial interval is 8–10 days and infectivity peaks during week 2 of illness. These factors allow little time for instituting the isolation and quarantine interventions that were essential in controlling SARS.

Entry Screening of Air-travel Passengers during 2003 SARS Outbreak

In the 2003 SARS experience, data from 4 Asian locations and Canada indicated that body temperature–sensing devices did not detect anyone with SARS among >35 million entering travelers screened. Administration of health declarations (a questionnaire completed by the traveler to report health information, e.g., symptoms and history of exposure) to >45 million entering travelers detected 4 SARS cases. At least 31 million health alert notices were distributed to entering international travelers in several countries, but follow-up information is limited. Mainland China reported the distribution of 450,000 notices and detection of 4 SARS cases possibly linked to the notices. Thailand reported printing 1 million notices and detecting 24 cases directly linked to them (*26*). The 5 persons with SARS who entered Canada did not have signs or symptoms at international airports; Canadian authorities concluded that border screening for SARS was insensitive and not cost-effective and that surveillance allowing for early detection of imported cases was preferable (*27*).

The possible effect of entry screening for pandemic influenza has been estimated for the United Kingdom, with the assumption that exit screening is in place at international airports in countries with pandemic influenza. A mean of 9% of persons infected by influenza who were asymptomatic on departure would be estimated to develop influenza symptoms en route to the United Kingdom; the percentage would be higher during longer flights. Symptoms would develop in an estimated mean of 17%

(range 12%–23%) of infected persons traveling from Asian cities. Airplanes that arrive daily at 12 airports in the United Kingdom from the Far East have >12,000 seats; entry screening would fail to detect ≈83% of infected persons (*28*). Travelers arriving on connecting flights were not considered. In Taiwan during the 2003 SARS outbreak, 80,813 incoming air-travel passengers from affected areas were quarantined; 21 (0.03%) were diagnosed with suspected or probable SARS. None of these 21 cases had been detected by entry screening (*26*,*29*). Another modeling study from the UK Health Protection Agency suggests that reduction of air travel to and from affected areas, if implemented, must be almost total and nearly instantaneous to delay pandemic spread significantly (B. Cooper, pers. comm.).

Exit Screening of Travelers during SARS Outbreak

After WHO recommended exit screening of international travelers departing from affected areas on March 27, 2003, no additional spread of SARS through air travel was documented from countries with exit screening. This finding may reflect a deterrence effect, a generally low incidence of SARS cases, or both. Combined data from several countries indicate 1 case detected among 1.8 million departing passengers completing health questionnaires and no cases among 7 million persons who underwent thermal scanning on departure (*26*).

Measures To Limit Influenza Virus Transmission on Conveyances

Influenza has been transmitted on airplanes (*30*) and ships (*31*). In 1 cluster, influenzalike illness developed in 72% of passengers seated in an airplane that was on the ground for 3 h without ventilation and that held a person with symptomatic influenza (*9*). On a 75-seat aircraft, 15 passengers traveling with an influenza-infected person became ill. All 15 persons were seated within 5 rows of the index patient, and 9 were seated within 2 rows (*32*).

In a review of the Australian experience with pandemic influenza aboard ships in 1918 to 1919, a "Daily thermometer parade and removal of any person febrile or reporting sick (was) most thoroughly and efficiently carried out" (*17*). Despite these measures, examples were given of 3 ships with 89%, 46%, and 30%, respectively, of those onboard who were ill, which led to the "conclusion that neither inhalation, inoculation, nor isolation of the sick would stop an epidemic. . . . No administrative measure was successful in modifying the time factor of a shipboard epidemic, although there is some reason for believing that the measures employed were, by their combined influence, successful in reducing the potential volume of actual cases" (*17*).

Influenza outbreaks have been reported on cruise ships during international voyages (*31*). A large summertime outbreak involved both international travelers and crew during 3 cruises of 1 ship. Control measures included surveillance, isolation of ill crew, immunization of the crew, and use of antiviral drugs for treatment and prophylaxis of crew and passengers (*31*,*33*).

During the 2003 SARS outbreak, the disease was transmitted on and spread internationally via aircraft. The most extensive investigation included 3 flights on which an index passenger had SARS; on 1 of these flights, 22 (18.3%) of 120 other passengers and crew became infected. A higher risk was noted for passengers seated near the index patient, but most passengers who became infected were seated farther away, even though their individual risk was lower (*34*). In most other investigations, no transmissions were documented, although the investigations were limited (*26*).

Discussion

The effectiveness of nonpharmaceutical public health interventions in affecting the spread of pandemic influenza depends on transmission characteristics of the virus. If a substantial proportion of transmission occurs during the incubation period or during asymptomatic infection, the population impact of health screening and case-patient isolation will be diminished. The age distribution of patients is also important: if children play a central role in initial community transmission, school closure would likely be more effective. Since a new pandemic subtype might have different transmission characteristics than previous subtypes, these characteristics and associated illness patterns must be assessed in the field as soon as human-to-human transmission begins. Monitoring over time is also needed to assess possible changes as the virus becomes more adapted to human hosts.

WHO has developed recommendations to provide guidance until transmission characteristics can be determined. The recommendations are based on limited information, including virologic data from seasonal epidemics and volunteer studies rather than pandemics, in which shedding and transmission may be more intense and prolonged because of lack of population immunity. These data indicate that influenza viral shedding in the upper respiratory tract (and presumably also infectiousness) is correlated with fever and the severity of respiratory symptoms in both adults and children. The importance of transmission from infected persons during the incubation period or from persons with asymptomatic infection is uncertain but appears to be substantially less than from symptomatic persons. The principal difficulties in using nonpharmaceutical interventions to reduce influenza transmission among

humans include the peak infectivity early in illness and the short incubation period, which both result in a short serial interval between related cases. Recent reports suggest that the 1918 virus may have been less transmissible than previously thought (R_0 1.8–3), although whether public health interventions in 1918 might have affected these estimates is uncertain. If a novel human influenza subtype behaves in a manner similar to the pandemic virus of 1918–1919, available information supports the use of nonpharmaceutical interventions to delay or contain transmission during WHO phases 4 and 5 (limited human-to-human transmission) and use of different interventions to reduce the impact in phase 6 (pandemic phase) (*2*,*3*).

At the international level, experience in past influenza pandemics indicates that screening and quarantine of entering travelers at international borders did not substantially delay introduction, except in some island countries. Similar policies, even if they could be implemented in time and regardless of expense, would doubtfully be more effective in the modern era of extensive international air travel. WHO instead recommends that travelers receive health alert notices, although entry screening may be considered when the host country suspects that exit screening at the traveler's point of embarkation is suboptimal; in geographically isolated, infection-free areas (e.g., islands); and where a host country's internal surveillance capacity is limited (*2*).

WHO recommends consideration of exit screening by health declaration and temperature measurement for international travelers departing countries with human infection at phases 4, 5, and 6. Exit screening in affected countries is a better use of global resources: fewer persons would need to be screened, the positive predictive value for ill persons detected would be higher, and transmission on conveyances, such as aircraft, would be reduced. Exit screening is disruptive and costly, however, and will not be fully efficient as influenza viruses can be carried by asymptomatic persons who will escape detection during screening (*2*,*3*). As was true for SARS, the principal focus of WHO-recommended nonpharmaceutical interventions is not at international borders but at national and community levels (*4*).

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Address for correspondence: David M. Bell, Office of Strategy and Innovation, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D28, Atlanta, GA 30333, USA; fax: 404-639-5172; email dbell@cdc.gov

Nonpharmaceutical Interventions for Pandemic Influenza, National and Community Measures

World Health Organization Writing Group*[1]

The World Health Organization's recommended pandemic influenza interventions, based on limited data, vary by transmission pattern, pandemic phase, and illness severity and extent. In the pandemic alert period, recommendations include isolation of patients and quarantine of contacts, accompanied by antiviral therapy. During the pandemic period, the focus shifts to delaying spread and reducing effects through population-based measures. Ill persons should remain home when they first became symptomatic, but forced isolation and quarantine are ineffective and impractical. If the pandemic is severe, social distancing measures such as school closures should be considered. Nonessential domestic travel to affected areas should be deferred. Hand and respiratory hygiene should be routine; mask use should be based on setting and risk, and contaminated household surfaces should be disinfected. Additional research and field assessments during pandemics are essential to update recommendations. Legal authority and procedures for implementing interventions should be understood in advance and should respect cultural differences and human rights.

This article is the second of a 2-part series that summarizes the scientific basis for nonpharmaceutical public health interventions recommended by the World Health Organization (WHO) to contain or reduce transmission of pandemic influenza caused by a novel human influenza subtype; it is designed to be read in conjunction with the recommendations (*1*), which are intended as guidance and not formal WHO advice (Appendix 1, available online at http://www.cdc.gov/ncidod/EID/vol12no01/05-1371_app1.htm) (*2*). The evidence base for recommendations is limited, consisting primarily of historical and contemporary observations, rather than controlled scientific studies. The first part of this series summarized the transmission characteristics of influenza viruses and the basis for interventions to reduce international spread (*3*). This second part addresses measures at the national and community levels that are intended to reduce exposure of susceptible persons to the novel virus. The observations that pandemics do not infect all susceptible persons in the first wave and that subsequent waves occur suggest that preventing disease by reducing exposure is an achievable objective (*3*). By limiting exposure, people who are not infected during the first wave may have an increased chance of receiving virus-specific vaccine as it becomes available. In addition, if the virus becomes less virulent over time, persons who fall ill in subsequent waves may have milder illnesses. This article does not address public communication or infection-control measures for patient care (*4*,*5*).

Measures To Reduce Spread within Populations

Isolation of Patients and Quarantine of Contacts

Community Level

Reports from many countries indicate that mandatory case reporting and isolating patients during the influenza

*World Health Organization, Geneva, Switzerland

[1]The writing group was established by request of the WHO Global Influenza Programme. It consisted of the following persons: David Bell, Centers for Disease Control and Prevention, Atlanta, Georgia, USA (coordinator); Angus Nicoll, European Centre for Disease Prevention and Control, Stockholm, Sweden, and Health Protection Agency, London, United Kingdom (working group chair); Keiji Fukuda, WHO, Geneva, Switzerland; Peter Horby, WHO, Hanoi, Vietnam; and Arnold Monto, University of Michigan, Ann Arbor, Michigan, USA. In addition, the following persons made substantial contributions: Frederick Hayden, University of Virginia, Charlottesville, Virginia, USA; Clare Wylks and Lance Sanders, Australian Government Department of Health and Ageing, Canberra, Australian Capital Territory, Australia; and Jonathan Van Tam, Health Protection Agency, London, United Kingdom.

pandemic of 1918 did not stop virus transmission and were impractical. In Canada, the medical officer of health for the province of Alberta concluded that forced home isolation of patients, posting signs on houses, and "quarantine" (details unspecified) captured only ≈60% of patients in the community because of diagnostic difficulties involving mild cases and failure to notify cases to authorities. As the medical officer noted, "many citizens regarded the placard [sign outside the quarantined person's house] as an injustice, either because they did not believe the diagnosis justified, or because their neighbors were alleged by them to be avoiding quarantine by concealment or evasion… Charges of discrimination were frequently made against the health department" (*6*).

In the Australian state of New South Wales, compulsory reporting was deemed helpful to identify the first introduction of cases into a community. However, once the number of cases grew, reporting cases was not useful or feasible. Also, mild cases were not reported. Compulsory home isolation (which automatically followed reporting) prevented neighbors from bringing needed assistance and was replaced by requesting patients to remain at home (*7*). The reports do not assess the potential impact that requests for ill persons to remain at home voluntarily could have on the reduction of disease within the community.

Closed Settings

In closed settings (e.g., military barracks and college dormitories), early identification and isolation of patients in 1918 usually did not completely stop virus transmission but appeared to decrease attack rates, especially when supplemented by restrictions on travel to and from the surrounding community (*8*). In 1 report, 2 sections (A and B) of the student army training corps at the University of Chicago were housed in similar dormitories and fraternity houses, but they had separate classrooms and eating places and no formal contact with each other. In section A, the men received frequent instructions to report illness; all ill persons with "simple colds" or suspected influenza were immediately isolated in hospitals or sent home. In section B, "more or less close contact between sick and well members" was maintained for several days. Lectures and classes were held as usual. From October 17 to November 8, 1918, a total of 26 of 685 men in section A had influenza (attack rate 39/1,000), which was one tenth the attack rate for section B (398/1,000, 93/234 men). New cases ceased in section B after daily inspection and patient isolation were implemented, but these measures were taken late in the epidemic. Among 82 other students living at home or in boarding houses, 7 became ill with influenza (*9*). Similarly, an Australian Quarantine Service review of ship epidemics in 1918 and 1919, including ships quarantined at ports of entry, indicates that daily temperature checks and immediate isolation of patients did not completely prevent transmission but may have reduced the number of cases (*3*).

Reports from several countries (e.g., Australia, Canada, British-occupied Togo) refer to "isolation of contacts" (the preferred modern terminology is isolation of patients and quarantine of contacts) in 1918 and 1919. Details are unclear, but these reports imply that contacts were confined at home. Such measures were consistently described as ineffective and impractical (*6*,*7*,*10*)

Some of the lessons learned from the 2003 severe acute respiratory syndrome (SARS) epidemic can be applied to influenza, including the success of public campaigns to encourage self-recognition of illness, telephone hotlines providing medical advice, and early isolation when potential patients seek health care. Thermally scanning intercity travelers was inefficient in detecting cases. Early isolation of patients and quarantine of contacts successfully interrupted SARS transmission, but influenza's shorter serial interval and earlier peak infectivity, plus the presence of mild cases and possibility of transmission without symptoms, suggest that these measures would be considerably less successful than they were for SARS (*3*,*11*,*12*).

Social Distancing Measures

Avoiding Crowding

A WHO consultation in 1959 concluded that the 1957 influenza pandemic tended to appear first in army units, schools, and other groups where contact was close. Also noting the reduced incidence in rural areas, the consultation suggested that avoiding crowding could reduce the peak incidence of an epidemic and spread it over many, rather than a few, weeks (*13*).

Closing Schools and Childcare Centers

A 1959 WHO consultation concluded, "In the Northern hemisphere at least, the opening of schools after the summer holidays seems to have played an important role in initiating the main epidemic phase" (*13*). Despite the propensity of influenza epidemics to be amplified in primary schools (*14*), data on the effectiveness of school closures are limited. Apparently no data or analyses exist for recommending illness thresholds or rates of change that should lead to considering closing or reopening schools.

During a 2-week teachers' strike during an influenza epidemic in Israel in 2000, significant decreases were seen in the rates of diagnoses of respiratory infections, medication purchases, and other parameters for children 6–12 years of age; when school reopened, rates for these parameters rose again. The study did not report on illness in family members (*15*). In 21 regions of France from 1984 to 2000, a temporal relationship was reported between school

holidays and a decrease in the incidence of influenza diagnoses by general practitioners 10–20 days later and the daily death rate 30–40 days later, although the time delay raises the question of whether outbreaks may have been subsiding on their own (*16*).

On a small island in the United States in 1920, the single public school was a focal point for the spread of influenza, and a report from that period concluded that "prompt closure of the school would probably not have prevented the epidemic, but might have delayed it" (*17*). School closure might be less effective in some urban areas than in rural areas because urban children can more easily meet elsewhere: in 1918, more influenza cases developed among pupils in a Chicago school after a holiday than when schools were in session (*9*). In Connecticut, the 3 largest cities (Bridgeport, Hartford, and New Haven) kept schools open under "close medical supervision," and their death rates were reportedly lower than those in some Connecticut cities (New London and Waterbury) that closed their schools (*8*).

Universal influenza vaccination of children is controversial, but its use has provided data that help assess the potential effect of reducing transmission by schoolchildren. For example, in 1968–1969, when 86% of its schoolchildren were vaccinated against influenza, the small town of Tecumseh, Michigan, had one third the illness rate of nearby towns where children were not vaccinated (*18*). In Japan, when most schoolchildren were vaccinated against influenza (1962–1987), excess death in the entire population decreased 3- to 4-fold and rose again when the program was discontinued (*19*).

Simultaneous Use of Multiple Measures

Influenza and other respiratory viral infections apparently declined in Hong Kong during the 2003 SARS epidemic, as determined on the basis of a review of viral diagnostic laboratory records (*20*). Public health interventions included closing schools, swimming pools, and other public gathering places; cancelling sports events; and disinfecting taxis, buses, and public places. A high percentage of people wore masks in public and washed hands frequently, and in general, much less social mixing occurred.

Reports from the 1918 influenza pandemic indicate that social-distancing measures did not stop or appear to dramatically reduce transmission, but research studies that might assess partial effectiveness are apparently unavailable. For example, in Lomé, British-occupied Togo, case-patients, suspected case-patients, and contacts were isolated; traffic was halted; schools and churches were closed; public meetings were banned. Despite these and other measures, influenza was well established in Lomé by October (*10*). In Edmonton, Canada, isolation and quarantine were instituted; public meetings were banned; schools, churches, colleges, theaters, and other public gathering places were closed; and business hours were restricted without obvious impact on the epidemic (*21*,*22*). In the United States, a comprehensive report on the 1918 pandemic concluded that closing schools, churches, and theaters was not demonstrably effective in urban areas but might be effective in smaller towns and rural districts, where group contacts are less numerous (*8*).

Measures for Persons Entering or Exiting an Infected Area

In Australia in 1919, political tensions arose among state governments and between states and the national government as individual states sought to protect themselves. Issues included delayed disease reporting by the initially affected state, controls at interstate borders, resistance to quarantine measures, impoundment of the transcontinental train in the state of Western Australia, and conflict between national and state authorities in the Australian federal system (*23*).

Specific details were recorded by the State of New South Wales (NSW) (*24*): "After the first case was diagnosed in Sydney (capital of NSW State) …and determined to have come from (the) adjacent (state of) Victoria, measures were taken by New South Wales at the interstate border to prevent importation of additional cases. These included at first, prohibition of all inbound land traffic, later replaced by quarantine detention camps at which inbound travelers were required to remain at first 7, later 4 days. Also ships from Victoria State were required to anchor in Sydney harbor for 4 days, after which disembarking persons were medically inspected. After Sydney had nevertheless become severely affected, (unspecified) restrictions on traveling out of Sydney were also imposed." The report states that any benefits of land quarantine or interstate or intrastate travel restrictions were "very meager."

In Canada in 1918, one report noted, "Many small towns attempted to isolate themselves with complete quarantines, reminiscent of medieval attempts to stave off plague, in which no one was allowed to enter or leave town. No one was allowed to buy railway tickets to these towns and passengers were barred from disembarking at them. The Canadian Pacific Railway reported 40–45 towns closed in the province of Manitoba during the height of the epidemic; the Canadian Northern line bypassed 15 more. The Alberta Provincial Police guarded roadblocks on major highways in the Province of Alberta in an effort to keep influenza from reaching three prairie municipalities. These measures were nonetheless 'lamentably inefficient in checking the spread of the disease.' Quite simply, isolating individuals and families or quarantining entire communities did not work" (*6*,*21*,*22*).

In the United States, some towns in Colorado and Alaska implemented measures, such as a 5-day quarantine on entering travelers, to exclude infected people. Some towns apparently succeeded in escaping the disease, but others did not (*8*,*25*). In July 1921, an explosive outbreak of influenza occurred on the Pacific island of New Caledonia, a French territory. Authorities implicated a ship that had recently arrived at the capital city of Nouméa from Australia, where normal seasonal (winter) influenza cases were occurring. Illness spread rapidly in Nouméa and the southern portion of the colony, in part because of numerous gatherings in celebration of Bastille Day on July 14. However, authorities successfully prevented spread to the isolated northern third of the island. Travel by land to the north was prohibited, a measure that was facilitated by the lack of major roads to the area. Ships leaving Nouméa for the north were required to remain in quarantine for at least 48 hours before departure, and during that time, temperatures of passengers and crew were monitored (*26*).

Recent modeling studies have supported the use of quarantine measures in the unique circumstances of containing an emerging influenza subtype originating in rural Thailand as a supplement to geographically targeting antiviral drugs to the surrounding population. In 1 model, administering antiviral drugs to 90% of people in a 5-km radius within 2 days after detecting illness in 20 persons was estimated to contain a novel subtype with a basic reproduction number (R_0) of 1.5 (R_0 is the mean number of secondary cases generated by 1 infected person in a fully susceptible population). If prophylaxis were supplemented by closing 90% of schools and 50% of workplaces and reducing movement in and out of the affected area by 80%, the model predicted a 90% probability of containment if R_0 = 1.9 (*27*). These additional measures would help overcome shortcomings in case identification and treatment rates; the epidemic could be contained after <200 cases had been detected. Unsuccessful containment nevertheless delayed widescale spread by ≥1 month in the model. A second modeling study predicted that if every case-patient stayed at home and 70% of susceptible persons remained in their neighborhoods (but no antivirals were given), disease containment would be 98% if R_0 = 1.4 and 57% if R_0 = 1.7 (*28*). These estimates were based on the population structure and interaction dynamics in Thailand and apply to early detection of cases emerging in a rural area.

Personal Protection and Hygiene Measures

Wearing Masks in Public

Apparently no controlled studies assess the efficacy of mask use in preventing transmission of influenza viruses. During the 1918 influenza pandemic, mask use was common and even required by law in many jurisdictions. Skepticism arose, however; the medical officer of health for Alberta, Canada, noted that cases of disease continued to increase after mask use was mandated, and public confidence in the measure's efficacy gave way to ridicule (*6*).

In Australia, mask-wearing by healthcare workers was thought to be protective, and given evidence of transmission in a closed railway carriage, it was concluded that mask wearing "in closed tramcars, railway carriages, lifts, shops, and other in enclosed places frequented by the public had much to recommend it." However, mask-wearing in the open air, as initially required in Sydney, was later thought to be unnecessary (*24*).

In the United States, persons also wore masks as a protective measure. A report from Tucson, Arizona, noted that early measures included "…isolation of ill people, closure of schools, churches, theatres, etc. The epidemic worsened however. As weeks passed, criticism of the measures was expressed, most vocally by businesses losing money but also by religious and educational institutions. To allow some businesses to reopen, city officials ordered 'masks to be worn in any place where people meet for the transaction of necessary business' … (and later by) all persons appearing in public places. Within a few days, there was virtually universal compliance with mask wearing, but the epidemic was subsiding" (29).

During the SARS epidemic in 2003, 76% of Hong Kong residents reported wearing masks in public. As noted above, influenza virus isolation rates decreased, but since multiple measures were implemented, the contribution of mask use, if any, is uncertain (*20*). In case-control studies conducted in Beijing and Hong Kong, wearing masks in public was independently associated with protection from SARS in a multivariate analysis. One study found a dose-response effect (*30*). Methodologic limitations of the studies (e.g., retrospective questionnaire design) limit drawing conclusions (*30*,*31*).

Hygiene and Disinfection

Recommendations for "respiratory hygiene/cough etiquette," such as covering one's mouth when coughing and avoiding spitting, have been made more on the basis of plausible effectiveness than controlled studies (*32*). As summarized in part 1 of this article, influenza virus can remain viable on environmental surfaces and is believed transmissible by hands or fomites (*3*). Most, but not all, controlled studies show a protective effect of handwashing in reducing upper respiratory infections (Appendix 2, available online at http://www.cdc.gov/ncidod/EID/vol12no01/05-1371_app2.htm). Most of the infections studied were likely viral, but only a small percentage were due to influenza (*33*). No studies appear to address influenza specifically. In addition, only 1 study (in Pakistan) has been conducted on the effect of handwashing on severe

disease (*34*). Most studies have been in care or institutional settings and involve children; the few involving adults were of college students and military recruits. Antibacterial handwashing products do not offer an advantage over soap and water. In the SARS outbreak in Hong Kong in 2003, a case-control study found that washing hands >10 times per day and "disinfecting living quarters thoroughly" (not otherwise defined and reported retrospectively by telephone) appeared to be protective in a multivariate analysis (*31*).

Discussion

The knowledge base for use in developing guidance for nonpharmaceutical interventions for influenza is limited and consists primarily of historical and contemporary observations, supplemented by mathematical models, rather than controlled studies evaluating interventions. Accordingly, WHO guidance is subject to revision based on additional information. Aside from transmission characteristics of the pandemic strain, which can be estimated but not completely known before a pandemic is under way, guidance for interventions at the national and community level depends on the phase of the pandemic, the severity of disease (a more virulent strain will justify more socially demanding measures), and the extent of transmission in the particular country and community. Animal sources of virus that has been linked to human infection should be controlled and human exposure to infected animals minimized (*35*). In phases 4 and 5 of the pandemic-alert period, which is characterized by limited and highly localized human-to-human transmission, aggressive measures to detect and isolate case-patients and to quarantine their contacts are recommended and should be accompanied by restrictions on movement in and out of affected communities and consideration of geographically targeted antiviral therapy. These measures, however, are considered much less likely to be feasible in an urban population (*1,3,27*).

The prediction from mathematical models that an emerging novel human influenza virus subtype might be containable at a point of origin in rural Southeast Asia in phases 4 and 5 through the targeted use of antivirals and application of public health measures was not intended to apply once a pandemic has begun or to address other situations (for example, when a pandemic strain enters into a new country at multiple loci) (*27,28*). After increasing and sustained transmission occurs in the general population of even 1 country (phase 6, pandemic period), eventual worldwide spread is considered virtually inevitable, and the public health response focus would shift to reducing impact and delaying spread to allow time for vaccine development and institution of other response measures. Part 1 of this article dealt with measures at the international level, but community-level measures outlined in this part of the article will likely have a greater effect, as was true for SARS in 2003. Over time, the changing conditions during a pandemic will require a change in the public health response and recommended interventions, and the need for such changes will present a difficult but critical communications challenge.

Field studies coordinated by WHO will be needed to assess virus transmission characteristics, amplifying groups (e.g., children vs. adults), and attack and death rates. Information on these factors will be needed urgently at the onset of a pandemic because the pandemic subtype may behave differently than previous pandemic or seasonal strains. Such studies will also be needed throughout the pandemic period to determine if these factors are changing and, if so, to make informed decisions regarding public health response measures, especially those that are more costly or disruptive.

Evidence and experience suggest that in pandemic phase 6 (increased and sustained transmission in the general population), aggressive interventions to isolate patients and quarantine contacts, even if they are the first patients detected in a community, would probably be ineffective, not a good use of limited health resources, and socially disruptive. During phase 6, ill persons should be advised to remain at home, if possible, as soon as symptoms develop (and their caregivers should be advised to take appropriate precautions [*5*]), but doing so would likely require financial and other support for those off work with illness. Measures to increase social distance should be considered in affected communities, depending on the epidemiology of transmission, severity of disease (case-fatality ratio), and risk groups affected. Nonessential domestic travel to affected areas should be deferred if large areas of a country remain unaffected, but enforcing domestic travel restrictions is considered impractical in most cases.

Handwashing and respiratory hygiene/cough etiquette (*32*) should be routine for all and strongly encouraged in public health messages; such practices should be facilitated by making hand-hygiene facilities available in schools, workplaces, and other settings where amplification of transmission would be expected. WHO has recommended that mask use by the public should be based on risk, including frequency of exposure and closeness of contact with potentially infectious persons; routine mask use in public places should be permitted but not required. This recommendation might be interpreted, for example, as supporting mask use in crowded settings such as public transport. The use of masks or respirators, as well as other precautions, for occupationally exposed workers also depends on risk and is beyond the scope of this review (*4,5*). Disinfection of household surfaces likely to be contaminated by infectious secretions appears worthwhile, but no evidence supports

the efficacy of widespread disinfection of the environment or air. The legal authority and procedures for implementing interventions should be understood by key personnel before a pandemic begins, and all such measures should respect cultural differences and human rights (*1*,*36*).

The need is urgent for additional research on transmission characteristics of influenza viruses and the effectiveness of nonpharmaceutical public health interventions. Such research should include epidemiologic and virologic studies and field assessments of effectiveness and cost, supplemented by modeling studies and historical inquiry. Such research could be undertaken during epidemics of seasonal influenza, and some research investment now being devoted to influenza should be dedicated to this end. Research needs include evaluating the effectiveness of mask use and cough etiquette and evaluating interventions in terms of cases detected and prevented, cost, and effectiveness in alleviating public concerns. Research is also needed to identify ways to make quarantine and other restrictions more focused and less burdensome for individual persons and societies and to assess how "leaky" restrictions can be and still be effective. Improved methods are also needed to communicate with essential partners and the public. Finally, improved informatics capabilities would allow outbreaks to be monitored and interventions to be assessed in real time to meet the needs of all who will help control future pandemics.

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Address for correspondence: David M. Bell, Office of Strategy and Innovation, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D28, Atlanta, GA 30333, USA; fax: 404-639-5172; email: dbell@cdc.gov

Economics of Neuraminidase Inhibitor Stockpiling for Pandemic Influenza, Singapore

Vernon J. Lee,* Kai Hong Phua,† Mark I. Chen,* Angela Chow,‡ Stefan Ma,‡ Kee Tai Goh,‡ and Yee Sin Leo*

We compared strategies for stockpiling neuraminidase inhibitors to treat and prevent influenza in Singapore. Cost-benefit and cost-effectiveness analyses, with Monte Carlo simulations, were used to determine economic outcomes. A pandemic in a population of 4.2 million would result in an estimated 525–1,775 deaths, 10,700–38,600 hospitalization days, and economic costs of $0.7 to $2.2 billion Singapore dollars. 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. These models can help policymakers weigh the options for pandemic planning.

Ten percent of the world's population and 20% of the population of tropical Singapore are infected with influenza virus annually (*1*,*2*). Amid growing concern about influenza pandemics, national preparedness plans have become essential. In a pandemic hastened by globalization, vaccination is not a viable initial solution because vaccine production requires an estimated 6 months (*1*,*3*). Instead, neuraminidase inhibitors are influenza-specific antiviral agents that figure strongly in preparedness plans. Many nations are acquiring stockpiles of these drugs because of their effectiveness in influenza treatment and prophylaxis (*4*).

Studies have compared the cost-effectiveness of vaccination versus treatment with antiviral agents (*5*–*7*), but only l study has examined the cost-effectiveness of prophylaxis (*8*). We provide further comparison of the economic outcomes of prophylaxis or treatment with antiviral agents to provide national planners with optimal strategies.

Methods

This study used a decision-based model (Figure 1) to perform cost-benefit and cost-effectiveness analyses for stockpiling antiviral agents in Singapore. Oseltamivir was the drug of choice because of its safety profile (*9*,*10*) and available data on influenza prophylaxis and treatment (*11*,*12*). The model compared 3 strategies: supportive management (no action), early treatment of clinical influenza with oseltamivir (treatment only), and prophylaxis in addition to early treatment (prophylaxis). Costs were assigned to each outcome, and probabilities at each node were aggregated as population rates for calculating overall costs

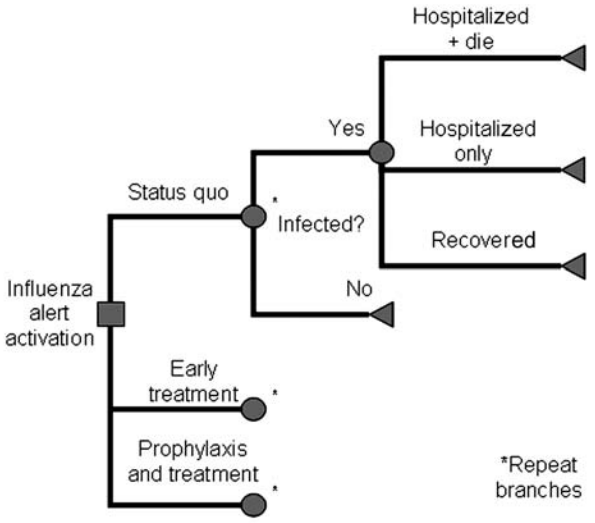

Figure 1. Decision-based model for strategies during pandemic influenza.

*Tan Tock Seng Hospital, Singapore; †National University of Singapore, Singapore; and ‡Ministry of Health, Singapore

for each outcome. Decision branches were similar for each strategy, but probabilities at individual nodes differed.

Cost-benefit analyses were used to compare treatment-only and prophylaxis strategies to taking no action. These analyses included direct and indirect economic costs, such as the cost of death. However, quantifying the societal cost of death is difficult, and cost-effectiveness analyses based on cost per life saved by treatment only and prophylaxis, compared to no action, were included. The model was run by using Excel spreadsheets (Microsoft Corp, Redmond, WA, USA); details are shown in the Appendix and on Tan Tock Seng Hospital's website (http://www.ttsh.com.sg/doc/Pandemic%20influenza%20in%20Singapore%20-%20economic%20analysis%20of%20treatment%20and%20prophylaxis%20stockpiling%20strategies.pdf). Costs are represented in 2004 Singapore dollars (2004 exchange rate, USD$1 = SGD$1.6908).

Pandemic influenza is unpredictable: uncertainties surround its occurrence and outcomes (*13*). Excess deaths in annual epidemics occur mostly in the elderly (*14*), but the 1918–1919 Spanish flu pandemic had higher death rates among adults (*15*). To account for such uncertainties, the input variables were modeled as triangular distributions centered on base values, with ranges corresponding to minimum and maximum values (Table 1). Sensitivity analyses, including 1-way analysis, were conducted to identify variables of highest impact and the outcome's sensitivity to treatment and prophylaxis stockpiles. Monte Carlo simulation analyses were performed to determine outcomes under different scenarios.

Treatment stockpiles, based on proportions of the population, are used on all influenzalike-illness cases, from pandemic plan activation until the pandemic ceases or the stockpile is depleted, whichever comes first. Analysis was conducted to determine the proportion of untreated influenza patients and simulation iterations with complete coverage, by stockpile levels. Further analysis was then performed for prophylaxis stockpiles where prophylaxis, by weeks, is given to the population over and above treatment requirements.

Input Variables

Input variables are shown in Table 1. Conservative values favoring no action were used to justify alternative strategies. The study was conducted on Singapore's 2004 midyear population of 4,240,300 (*16*), divided into 3 age groups, each consisting of 2 risk groups (low and high risk, according to underlying medical conditions predisposing the patient to influenza complications), for a total of 6 groups that represented differing infection outcomes and drug responses (*13*).

The clinical attack rates during the 1918 and 1957 pandemics were 29.4% and 24%, respectively (*23*), and attack rates in Singapore during the 1967 pandemic were 12.8%–36.4% (*22*). This study assumed a base clinical attack rate of 30% (range 10%–50%), corresponding to rates in other studies (*4*,*13*,*24*).

Case-fatality rates were derived from Singapore's excess deaths from interpandemic influenza; hospitalization and death were assumed to occur only in clinical influenza. To reflect hospitalization rates in relation to case-fatality rates, both rates were correlated. For outpatient visits, clinical influenza patients were assumed to seek medical care and take medical leave. However, some patients may not be treated effectively within 48 hours of infection, and they were assumed not to benefit from treatment.

For pandemic duration, influenza activity in tropical climates commonly rises above the baseline for ≥12 weeks (*31*,*33*), compared to 6 weeks in temperate climates (*34*). This study assumed a 12-week pandemic duration base value with a range from 6 weeks (average temperate duration) to 24 weeks (assumed vaccine development).

Individual economic value was calculated from the net present value of future earnings for average-aged persons in the respective age groups, adjusted for age. Other costs included were hospitalizations and work days lost; all costs were standardized to 2004 Singapore dollars.

Oseltamivir

This study relied on international studies on oseltamivir. Oseltamivir has a good safety profile with insignificant rates of severe adverse events and drug withdrawal (*9*). Costs from side effects were thus assumed to be insignificant compared to costs for pandemic illness and deaths. The known safe administration duration of 8 weeks represents only studied durations (*35*). Extension is assumed possible, and the model included up to 24 weeks' prophylaxis. Oseltamivir trials have lacked the power to detect mortality reductions because influenza deaths in trials are rare (*14*), and wide ranges were used to account for uncertainty. Oseltamivir is also less effective in the elderly (*24*). Immunity after prophylaxis among those without clinical infection was assumed to be 35%, as shown during an influenza study in which 38% of study participants on prophylaxis had serologic infection but no clinical infection (*12*). Oseltamivir's pharmacologic action is selective and is assumed to be inactive against noninfluenza illnesses.

Stockpile use depends on the probability of an influenza pandemic occurring. Antigenic shifts and reappearances of past variants were estimated to have pandemic potential every 8–10 years (*31*,*32*). Using oseltamivir's shelf-life of 4 years and patent expiration in 2016, the model assumed a conservative base value of 2.25 stockpile cycles before use (range 1–3.5 cycles) to account for significantly reduced costs after patent expiration. The model assumed that all unused stockpiles are lost.

Table 1. Input variables used in analysis*†

Input variables	Age ranges, y ≤19	20–64	≥65	Sources
Average age	10	40	73	*16*
Population, ×1,000 persons	999.2	2,962.5	278.6	*16*
Low risk, %	90	89.7	63.3	
High risk, %‡	10	10.3	36.7	*17–20*
Baseline influenzalike illness rate, cases/wk	7,686	19,940	750	*2,21*
Influenza clinical attack rate, % (range)	30 (10–50)	30 (10–50)	30 (10–50)	*4,13,22,23*
Case-fatality rate/100,000§				Ministry of Health, *4,13,24*
Low risk	5 (1–12.5)	6 (1–9)	340 (28–680)	
High risk	137 (12.6–765)	149 (10–570)	1,700 (276–3,400)	
Earnings lost per death, $¶	1,909,092	1,780,027	187,301	*16,25*
Hospitalization rate/100,000 infected#				Ministry of Health
Low risk	210 (42–525)	72 (12–108)	1,634 (135–3,268)	
High risk	210 (100–1,173)	234 (16–895)	2,167 (352–4,334)	
Average length of hospital stay, d	3.88 (2.3–9.2)	4.61 (3.2–11.8)	6.20 (4.6–13.4)	*13,24,26*
Average additional days lost	2 (1–3)	2 (1–3)	2 (1–3)	Local physicians
Hospital cost, $/d	342	342	342	Ministry of Health
Value of 1 lost day, $**	108	166/108	108	Ministry of Health, *25*
Outpatient				
Days lost from outpatient influenza	3 (1–5)	3 (1–5)	3 (1–5)	*9,13,23,27*
Consultation and outpatient treatment cost, $	40	40	40	Local physicians
Value of 1 lost day, $**	108	166	108	Ministry of Health, *25*
Treatment with oseltamivir				
Sought early medical care, %	70 (50–90)	70 (50–90)	70 (50–90)	*13,28*
Case-fatality rate reduction, %	70 (50–90)	70 (50–90)	30 (20–90)	*24,29*
Hospitalization rate reduction, %	60 (50–90)	60 (50–90)	30 (20–90)	*11,24*
Lost days gained, d	1.0 (0.1–2.0)	1.0 (0.1–2.0)	1.0 (0.1–2.0)	*7,9,24,28*
Treatment cost, $ per course	31	31	31	Ministry of Health
Prophylaxis with oseltamivir				
Efficacy of prophylaxis, %	70 (50–90)	70 (50–90)	70 (50–90)	*12,30*
Immunity after prophylaxis, %	35 (20–50)	35 (20–50)	35 (20–50)	*12,30*
Prophylaxis cost, $/wk	21.7	21.7	21.7	Ministry of Health
No. stockpile cycles to pandemic	2.25 (1–3.5)	2.25 (1–3.5)	2.25 (1–3.5)	*31,32*
Pandemic duration, wk		12 (6–24)		*32–34*
Treatment stockpile, % of population††		10–100		
Prophylaxis stockpile, wk††		2–24		

*All healthcare costs are in 2004 Singapore dollars and were compounded by using the consumer price index for Singapore (*16*).
†Base-case values are given with the range used for analysis given in parentheses, where applicable. Input variables were modeled as triangular distributions centered on base values; minimum and maximum values are given by extreme values in ranges.
‡High risk includes asthma, chronic obstructive pulmonary disease, heart disease, and diabetes patients.
§Based on deaths among those with clinical influenza.
¶Average present value of future earnings lost per death of a person of average age in the age group.
#Rate is based on hospitalizations among those with clinical influenza. Ranges were calculated based on a factor of the base cases versus the death rate.
**$166 for lost work day, $108 for unspecified days lost (taking care of ill child or elderly person), and additional days lost after hospitalization.
††The treatment and prophylaxis stockpiles are decision variables, and the analyses were performed for a range of values to determine the preferred outcomes.

Results

If no action were taken during a pandemic, the mean number of simulated deaths in Singapore would be 1,105 (5th and 95th percentiles of 525 and 1,775), with mean hospital days of 23,098 (10,736, 38,638). The mean economic cost would exceed SGD$1.43 billion (0.73, 2.19), and 78% of all deaths would occur in groups at high risk. From the sensitivity analyses, the outcome was most sensitive to changes in attack rate and case-fatality rate reduction with treatment and was sensitive to the variables of treatment and prophylaxis stockpiles.

Table 2 shows the cost and outcomes of various treatment stockpiles; each shelf-like cycle of the stockpile (which is 4 years, after which the drug has to be repurchased) costs SGD$13.1 million for 10% of the population. Stockpiles of <20% did not provide complete coverage in any simulated iterations, while stockpiles of >60% always provided complete coverage. The maximal mean economic benefit of SGD$414 million occurred at a 40% stockpile with 418 lives saved.

The population cost-benefit and cost-effectiveness outcomes from the Monte Carlo simulation analyses are

Table 2. Cost and outcomes with changes in treatment stockpile*†

% stockpile	Cost of stockpile (1 cycle, million $)	Overall % untreated influenza cases	% iterations with complete treatment	Lives saved	Overall benefit over no action (million $)
No action	NA	100	0	Deaths: 1,105 (525, 1,775)	Cost: 1,430 (730, 2,193)
10	13.1	89.1	0	49 (18, 108)	24 (–4, 73)
20	26.3	42.0	0	249 (128, 412)	224 (103, 385)
30	39.4	9.0	15	386 (185, 645)	385 (165, 619)
40	52.6	0.01	55	418 (185, 730)	414 (145, 759)
50	65.7	<0.01	90	422 (185, 744)	399 (122, 761)
60	78.9	0	100	422 (185, 744)	376 (98, 743)
70	92.0	0	100	422 (185, 744)	353 (76, 721)
80	105.2	0	100	422 (185, 744)	330 (52, 700)
90	118.3	0	100	422 (185, 744)	307 (26, 676)
100	131.4	0	100	422 (185, 744)	285 (4, 654)

*Mean values are shown with 5th and 95th percentiles in parentheses; NA, not available.
†All healthcare costs are in 2004 Singapore dollars.

shown in Table 3. The treatment-only strategy provided the best overall economic benefit, and the no-action strategy was dominated by the treatment-only strategy in cost per life saved.[1] Each additional week of prophylaxis costs SGD$92 million but reduced the overall economic benefit. Figure 2 shows that increasing the duration of prophylaxis increased lives saved. Lives saved from prophylaxis compared to treatment increased significantly only after prophylaxis of >4 weeks and increased steadily until 20 weeks; costs per life saved also increased.

Table 4 shows that treatment-only provided the greatest economic benefit across all groups. As prophylaxis duration increased, economic benefit decreased. However, for the 3 groups at high risk (Table 1), the mean overall economic benefit of up to 24 weeks' prophylaxis remained positive compared to that seen if no action was taken.

The simulated proportion of decisions with treatment only or 24 weeks' prophylaxis as the optimal outcome is shown in Figure 3. At case-fatality rates of 0.05% (similar to interpandemic epidemics), the decision always favored treatment-only. With increasing case-fatality rates, the decision increasingly favored prophylaxis and intersects between rates of 0.4% and 0.6%. Prophylaxis was always optimal in case-fatality rates of >1.5%. If no action was taken with a 5% case-fatality rate (the 1918 pandemic average) (*23*), 63,000 deaths, 1.5 million hospital days, and economic costs of SGD$112 billion would occur. Treatment-only saved 30,000 lives, benefited the economy by SGD$28–$84 billion, and required 780,000 hospital days. Twenty-four weeks of prophylaxis saved 50,000 lives, benefited the economy by SGD$46–$132 billion, and required 240,000 hospital days.

[1]"Dominate" is a term used in cost-effectiveness analyses and refers to a strategy that is both more efficacious and less costly than another strategy.

Discussion

The analyses suggest that treatment is always beneficial compared to no action and that the optimal treatment stockpile is 40%–60%: 40% maximizes economic benefits, while 60% maximizes treatment benefits. Compared to other strategies, treatment-only was the optimal economic strategy, while no action was always the least desirable option. Although treatment-only saved fewer lives than prophylaxis, stockpiling costs for treatment were lower. Prophylaxis was only economically beneficial compared with no action in subpopulations at high risk.

Substantial outcomes with prophylaxis occurred with durations of >4 weeks because shorter durations prolonged the pandemic, were insufficient for immunity, and did not cover the pandemic's peak. Increasing duration improved

Table 3. Cost-benefit and cost-effectiveness with changes in prophylaxis stockpile for the Singapore population*†

Strategy option	Stockpile cost (1 cycle, million $)	Lives saved compared with no action	Cost per life saved compared with no action ($100,000)	Benefit compared with no action (million $)
No action	Not applicable	Deaths: 1,105 (525, 1,775)	Not applicable	Cost: 1,430 (730, 2,193)
Only Rx‡	79	423 (183, 756)	38 (dominates§, 395)	379 (89, 734)
6 wk¶	631	492 (216, 870)	2,246 (811, 4,676)	–487 (–925, 48)
12 wk¶	1183	684 (286, 1,264)	3,193 (1,008, 6,788)	–1,188 (–1,934, –265)
18 wk¶	1735	850 (377, 1,442)	3,668 (1,358, 7,363)	–1,920 (–2,941, –783)
24 wk¶	2,287	903 (425, 1,509)	4,516 (1,828, 9,022)	–2,811 (–4,070, –1,384)

*Mean values are shown with 5th and 95th percentiles in parentheses.
†All healthcare costs are in 2004 Singapore dollars.
‡Only Rx refers to treatment only, without prophylaxis.
§Treatment-only dominates no action because treatment-only saves lives and is less costly overall.
¶No. of weeks of prophylaxis for the respective risk and age groups.

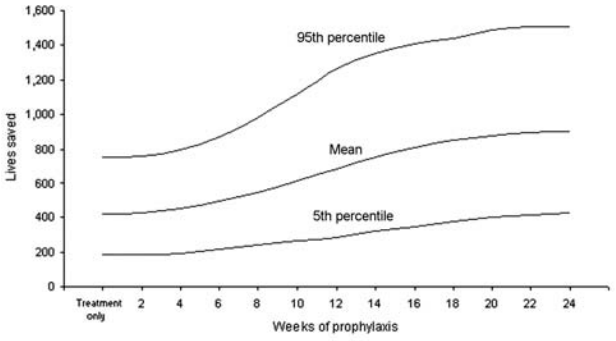

Figure 2. Lives saved compared with no action, by prophylaxis levels. Mean, 5th, and 95th percentiles based on Monte Carlo simulations are shown.

outcomes because it covered the pandemic's peak, but the improved outcomes tapered off after 20 weeks, resulting in a sigmoid curve (Figure 2).

In low-risk groups with low death and hospitalization rates, increasing prophylaxis duration decreased economic benefit and increased cost per life saved. In contrast, groups at high risk, who had higher death and hospitalization rates, were affected substantially by prophylaxis, resulting in overall benefits compared to taking no action. Elderly groups had the smallest populations but the highest risk levels and most deaths. However, their lower average future earnings compared to those of younger age groups resulted in lower overall benefits.

This study of pandemic outcomes in a tropical climate is similar to an Israeli study that compared treatment and prophylaxis strategies (*8*). Our study used local health outcome rates but did not include a ring prophylaxis strategy. Both studies found that oseltamivir treatment is economically beneficial, but in addition, our study showed that long-duration prophylaxis is beneficial for high-risk groups and high case-fatality pandemics.

Limitations of this study include the disregard for intangible costs, such as societal value of health; cost-utility analyses could address these costs. Also, indirect effects on national economy and world trade were not considered. For comparability, neither treatment nor prophylaxis was assumed to alter the pandemic's transmission dynamics. This assumption may be true if therapy is limited to small subpopulations, but it understates the benefits if infection is delayed until the pandemic is resolved or vaccine becomes available; it overestimates the benefits if the pandemic continues (*4*,*24*). Correlation between attack rates and pandemic duration was not accounted for, and all possible combinations were included.

Table 4. Outcomes by age and risk groups*

Risk and age group, y	Strategy option	Stockpile cost (1 cycle, million $)	Mean lives saved compared with no action	Mean cost per life saved compared with no action (million $)	Mean benefit compared with no action (million $)
Low risk, age <1–19	No action	NA	Deaths: 17	NA	Cost: 122
	Only Rx †	17	8	Dominates§	87
	12 wk ‡	251	11	41	–315
	24 wk ‡	485	14	70	–717
Low risk, age 20–64	No action	N/A	Deaths: 42	N/A	Cost: 507
	Only Rx	49	21	Dominates§	382
	12 wk	741	29	40	–808
	24 wk	1,433	36	73	–1,999
Low risk, age ≥65	No action	NA	Deaths: 185	NA	Cost: 57
	Only Rx	3	60	Dominates§	28
	12 wk	49	108	0.91	–43
	24 wk	95	148	1.3	–115
High risk, age >1–19	No action	NA	Deaths: 92	NA	Cost: 186
	Only Rx	2	45	Dominates§	94
	12 wk	28	63	1.0	83
	24 wk	54	78	1.8	66
High risk, age 20–64	No action	NA	Deaths: 220	NA	Cost: 443
	Only Rx	6	109	Dominates§	235
	12 wk	85	153	1.1	175
	24 wk	165	189	2.0	100
High risk, age ≥ 65	No action	NA	Deaths: 547	NA	Cost: 117
	Only Rx	2	179	Dominates§	44
	12 wk	29	321	0.17	24
	24 wk	55	438	0.25	0.1

*Mean values are shown, with all costs in 2004 Singapore dollars; NA, not applicable.
†Only Rx refers to treatment-only, without prophylaxis.
‡12 and 24 wk refer to number of weeks of prophylaxis for the respective risk and age groups.
§Treatment-only dominates no action because treatment-only saves lives and is less costly overall.

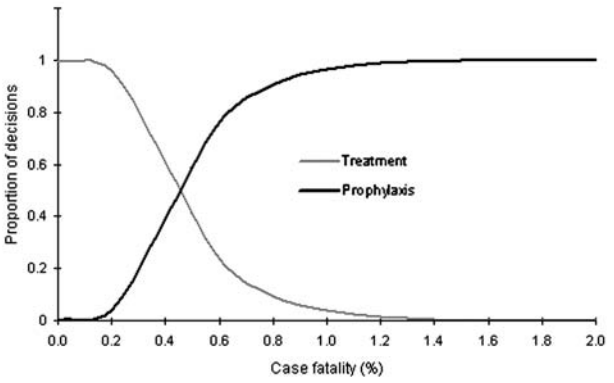

Figure 3. Proportion of decisions for treatment or 24 weeks prophylaxis, by case-fatality rate.

Policy Implications

Stockpiling is insurance in planning for pandemics with high case-fatality rates, in which more severe outcomes and higher risks demand higher premiums. Policymakers should consider lives saved even if economic costs outweigh incremental benefits. Prophylaxis of high-risk groups balances saving lives with economic benefits. Prophylaxis also reduces hospitalizations, which may otherwise overwhelm the healthcare system. Analysis of peak pandemic healthcare use is required to determine the effects of prophylaxis. Other options to reduce a pandemic's impact, including reducing influenza attack rates by quarantine or closing borders, should be considered as alternative strategies.

The current avian influenza (H5N1) outbreak in Asia, which has a high case-fatality rate, indicates the need for decisive action. Oseltamivir is effective against H5N1 and is used as treatment in Vietnam (*36*,*37*). Although resistance has been detected, resistant strains have poor infectivity (*37*). Prophylaxis with oseltamivir will reduce illness, deaths, and economic costs and may reduce spread. If avian influenza develops species crossover with case fatalities exceeding those of the 1918 Spanish influenza pandemic, then stockpiling for treatment and prophylaxis accrues substantial benefits.

The decision to stockpile requires predetermined objectives; noneconomic, moral, and ethical implications should be considered. Treatment-only maximizes economic benefits, while prophylaxis saves most lives. Policymakers have to act decisively, and determine the subpopulations to be given priority, to enable preparedness plans to succeed.

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Dr Lee is a preventive medicine physician with the Singapore Ministry of Defence, currently working at the Communicable Disease Centre, Tan Tock Seng Hospital, Singapore. His research interests include clinical cost-effectiveness, emerging infectious diseases management, and clinical process improvement.

Appendix

Details of the Equations Used in the Analysis

Antiviral stockpiles will be used on clinical influenza cases according to the pandemic distribution curve, assumed to be normally distributed (*4*). Baseline influenzalike illness rates are assumed to be constant.

Proportion Untreated

The population proportion with clinical influenza left untreated because of treatment stockpile deficiencies is calculated as follows:

No. of doses required = (influenzalike illness per week × pandemic duration) + no. of clinical influenza cases

Shortfall of doses for treatment = no. of doses required – no. of doses available

The proportion untreated is the shortfall of treatment doses matched to the number of case-patients who require treatment, according to the pandemic distribution curve.

Cost of Treatment and Prophylaxis

The cost of treatment was calculated as follows:

Total cost of treatment $\text{age}_{\text{risk group}}$ = cost of treatment per course × stockpile percentage × $\text{population}_{\text{age, risk group}}$

The cost of prophylaxis for 1 stockpile cycle was calculated as follows:

Total cost of $\text{prophylaxis}_{\text{age, risk group}}$ = cost of prophylaxis per week × no. weeks of prophylaxis × $\text{population}_{\text{age, risk group}}$

Cost of Outpatient Clinical Influenza

The medical cost of outpatient clinical influenza was calculated as follows:

Outpatient medical $\text{costs}_{\text{age, risk group}}$ = $\text{population}_{\text{age, risk group}}$ × attack rate × consultation and treatment cost

The cost of outpatient lost days was calculated by using work days lost for the adult population and unspecified days lost for the young and elderly populations, as follows:

Economic cost of outpatient $\text{lost days}_{\text{age, risk group}}$ = $\text{population}_{\text{age, risk group}}$ × attack rate × outpatient days lost × $\text{value of a day lost}_{\text{age, risk group}}$

Cost of Hospitalizations

The hospitalization cost for influenza-related complications was calculated by summing direct hospitalization cost with cost of additional days lost after hospitalization.

The direct hospitalization cost was calculated as follows:

Economic $\text{cost of hospitalization}_{\text{age, risk group}}$ = $\text{population}_{\text{age, risk group}}$ × attack rate × $\text{hospitalization rate}_{\text{age, risk group}}$ × length of stay

$_{\text{age, risk group}} \times$ (hospitalization cost + value of a day lost $_{\text{age, risk group}}$)

The cost from additional days lost was calculated as follows:

Economic cost of additional days lost after hospitalization = population $_{\text{age, risk group}} \times$ attack rate $\times$ hospitalization rate$_{\text{age, risk group}}$ $\times$ additional days lost$_{\text{age, risk group}} \times$ value of a day lost$_{\text{age, risk group}}$

Cost from Influenza Deaths

The cost from influenza deaths is calculated as follows:

Economic cost from influenza deaths = population$_{\text{age, risk group}}$ $\times$ attack rate $\times$ case-fatality rate$_{\text{age, risk group}} \times$ net present value of future earnings$_{\text{age, risk group}}$

Economic Calculations

For cost-benefit comparisons, the following equation is used:

Overall benefit = overall cost$_{\text{treatment only or prophylaxis}}$ − overall cost$_{\text{no action}}$

For the cost-effectiveness comparisons, the following equation is used:

Cost per-life-saved compared to no action = (cost excluding cost per life$_{\text{treatment-only or prophylaxis}}$ − cost excluding cost per life$_{\text{no action}}$) / (deaths$_{\text{no action}}$ − deaths$_{\text{treatment-only or prophylaxis}}$)

The individual costs that constitute the total costs are calculated for the strategies of no action, treatment-only, and prophylaxis as follows:

Overall cost$_{\text{no action, treatment-only, prophylaxis}} = \Sigma$ (population$_{\text{age, risk group}} \times$ probability of outcome$_{\text{clinical influenza, hospitalization, death}} \times$ cost of outcome$_{\text{clinical influenza, hospitalization, death}} \times$ effectiveness$_{\text{treatment-only, prophylaxis}}$) + cost of strategy$_{\text{treatment-only, prophylaxis}}$

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Address for correspondence: Vernon J. Lee, Block 802, Communicable Disease Centre, Moulmein Rd, Singapore 308433; fax: 65-6357-7465; email: vernonljm@hotmail.com

Estimating Influenza Hospitalizations among Children

Carlos G. Grijalva,* Allen S. Craig,*† William D. Dupont,* Carolyn B. Bridges,‡ Stephanie J. Schrag,‡ Marika K. Iwane,‡ William Schaffner,* Kathryn M. Edwards,* and Marie R. Griffin*

Although influenza causes more hospitalizations and deaths among American children than any other vaccine-preventable disease, deriving accurate population-based estimates of disease impact is challenging. Using 2 independent surveillance systems, we performed a capture-recapture analysis to estimate influenza-associated hospitalizations in children in Davidson County, Tennessee, during the 2003–2004 influenza season. The New Vaccine Surveillance Network (NVSN) enrolled children hospitalized with respiratory symptoms or fever and tested them for influenza. The Tennessee Emerging Infections Program (EIP) identified inpatients with positive influenza diagnostic test results through review of laboratory and infection control logs. The hospitalization rate estimated from the capture-recapture analysis in children <5 years of age was 2.4 per 1,000 (95% confidence interval 1.8–3.8). When NVSN estimates were compared with capture-recapture estimates, NVSN found 84% of community-acquired cases, EIP found 64% of cases in which an influenza rapid test was performed, and the overall sensitivity of NVSN and EIP for influenza hospitalizations was 73% and 38%, respectively.

Influenza is an important cause of acute respiratory infections and hospitalization in children (*1–10*). Since influenza may be indistinguishable from other respiratory and febrile illnesses, identification of infection requires diagnostic testing. Population-based studies report attack rates ranging from 15% to 42% in preschool and school children during typical outbreaks (*11*,*12*). However, defining the impact of influenza for more serious outcomes such as hospitalizations and deaths requires surveillance and testing of large populations, which may be expensive and time-consuming. Influenza surveillance systems can identify onset of disease activity, characterize viral isolates to help decide future vaccine composition, assess the impact of disease in different age and risk groups, and estimate vaccine impact (*4–6*,*13*,*14*). Identification of all cases of influenza or an unbiased sample of cases without regard to vaccination status is necessary to correctly measure disease impact and to assess vaccine effectiveness.

From 2003 to 2004, two independent population-based surveillance systems operated in Davidson County, Tennessee, to evaluate the impact of influenza disease in children. One prospectively tested samples from children <5 years of age who had been hospitalized with fever or respiratory symptoms. The other retrospectively identified hospitalizations for children with laboratory-confirmed influenza based on review of laboratory and infection control logs. Using data from the 2003–2004 influenza season independently generated by both systems for Davidson County residents <5 years of age, we applied a capture-recapture technique to obtain a better estimate of the total number of young children hospitalized with influenza.

Methods

The 2 surveillance systems used in Davidson County to assess the impact of influenza disease were the New Vaccine Surveillance Network (NVSN) and the Emerging Infections Program (EIP). The Centers for Disease Control and Prevention (CDC) established the NVSN in 1999 to evaluate the incidence of acute viral respiratory infections and to assess the impact of new vaccines and vaccination policies. Influenza surveillance in the NVSN has been conducted among children <5 years of age in the inpatient setting year round since August 2000. Three sites conduct active population-based surveillance, but only the Davidson County site was included for this study. Davidson County has an estimated population of 37,813

*Vanderbilt University School of Medicine; Nashville, Tennessee, USA; †Tennessee Department of Health, Nashville, Tennessee, USA; and ‡Centers for Disease Control and Prevention, Atlanta, Georgia, USA

children <5 years of age (2000 US Census). County residents <5 years of age hospitalized with respiratory symptoms or fever were enrolled 4 days per week and within 48 h of admission after informed consent was obtained. When a child was enrolled, a questionnaire was administered to parents, and 1 nasal and 1 throat swab specimen were collected from the child. These specimens were combined in a tube of veal infusion broth transport medium and delivered at ambient temperature within 1 to 2 h to the site research laboratory. Swab specimens are comparable to nasopharyngeal washes for influenza detection; however, swabs are more acceptable to families and less expensive to obtain (*15–18*).

Viral culture and reverse transcription–polymerase chain reaction (RT-PCR) were performed on these samples and medical charts were reviewed. To exclude nosocomial infections, NVSN excluded newborns who never left the hospital and those hospitalized in the previous 4 days. Similarly, children whose parents refused enrollment and those who were transferred from another surveillance hospital (to avoid double enrollment) were excluded. Children who were ill for >14 days did not meet our definition of acute respiratory illness, and those with fever and neutropenia were excluded because of logistic reasons. A child was considered to have influenza if the viral culture was positive or the RT-PCR result was positive on the initial test and 1 repeat test using a duplicate specimen aliquot. The results of these tests were not entered in the hospital chart and were not communicated to clinicians. NVSN performed surveillance at 3 hospitals that historically included at least 95% of all acute respiratory illness hospitalizations for children <5 years of age in Davidson County (*14*).

EIP, which was also organized and supported by CDC, was initially designed to estimate the impact of community-acquired invasive bacterial and foodborne infections through a population-based surveillance system (*19*). Because of unusual influenza activity during the 2003–2004 influenza season (*20*), EIP expanded its activities to conduct active, population-based surveillance for clinical laboratory-confirmed influenza hospitalizations in patients <18 years of age. For this analysis, only Davidson County data for children <5 years of age were included. EIP estimates the incidence of influenza hospitalizations by identifying hospitalized children with the diagnosis of influenza established by clinical laboratory testing. In Davidson County, in addition to those 3 hospitals where NVSN conducted surveillance, EIP included 7 additional hospitals that occasionally admitted Davidson County children. Hospitalized children <5 years of age with a clinical laboratory test result indicating influenza were identified and their charts were reviewed. For EIP, whether to test and which test to use were at the discretion of the attending physicians who were responsible for the child's medical care. Commercially available rapid tests, viral culture, immunofluorescence antibody staining, RT-PCR, immunohistochemical staining, and serologic analysis of paired acute-phase and convalescent-phase sera indicating a 4-fold increase in influenza antibody titer were the diagnostic techniques accepted by the EIP. A statement in the medical history that the child had a positive rapid test result for influenza performed in the outpatient setting was also acceptable. The EIP excluded children who were hospitalized >14 days after they tested positive for influenza and children whose symptom onset was >3 days after hospital admission.

A child enrolled as an influenza hospitalization by both NVSN and EIP was defined as a matched case. The identification of matched cases was determined retrospectively by comparing identified cases from the 2 systems and was based on name, date of birth, and date and place of hospitalization.

Institutional Review Boards (IRBs) of the participating hospitals and CDC approved NVSN surveillance. Since EIP influenza surveillance was considered a public health response program, it was exempt from IRB review and did not require informed consent of subjects or parents. This study was reviewed and approved by the Vanderbilt University IRB.

Statistical Analysis

We denoted as N the true total number of children <5 years of age hospitalized with influenza during the surveillance period in Davidson County. We estimated N by using the Petersen capture-recapture estimator (21), which we denoted $\hat{N}$ (Figure 1). The first surveillance system (NVSN) captured n1 cases from the total number of cases (N). The probability of capture is estimated by n1/N. The second system (EIP) captured n2 cases, including m2 cases that were already captured by the first system (recaptured or matched cases). The probability of being recaptured by the second system is estimated by m2/n2. When the probabilities of capture by 2 surveillance systems are independent, the probability of capture by the first system will equal the probability of recapture by the second. Equating our estimates of these probabilities and solving for N gives the Peterson estimator or $\hat{N} = n1 \times n2/m2$. This estimate assumes that the probability of being captured by 1 system does not affect the probability of being captured by the other, that the population is closed (the study population remained approximately constant and without significant migration during the study period), and that the ascertainment of influenza by the surveillance systems is valid (*21–25*).

Confidence intervals (CIs) for N were calculated using likelihood-ratio support intervals (*26*). The 95% CI for N

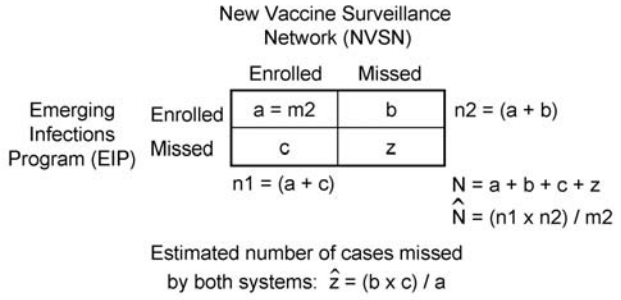

Figure 1. Capture-recapture estimation using data from 2 independent sources. The first surveillance system (New Vaccine Surveillance Network [NVSN]) captured n1 cases. The second system (Emerging Infections Program [EIP]) captured n2 cases, including m2 cases already captured by NVSN (matched cases). The Peterson estimator of N (total cases) is $\hat{N} = n1 \times n2/m2$. The Peterson estimate implies that the estimated number of cases missed by both systems (z) = (b x c)/(a); where b is the number of enrolled cases by the EIP only, c is the number of enrolled cases by the NVSN only, and a is the number of matched cases (m2) (21–25).

consisted of all population sizes for which the log-likelihood-ratio chi-square statistic was <3.84. Since NVSN attempted to identify all cases on surveillance days, the age distribution derived from this system likely represented the true age distribution of cases. This age distribution was applied to the capture-recapture estimated total cases to derive age-specific estimates for children <6 months, 6–23 months, and 24–59 months of age.

Data collected in preparing to establish NVSN showed that admission rates for acute respiratory infections were similar for study sampling and nonsampling days. Hospitalizations per 1,000 children for NVSN were estimated by weighting the observed number of enrolled hospitalizations to account for sampling 4 days a week (Sunday 7:00 a.m. to Thursday 7:00 a.m.) and nonenrollment. This weighting factor has 2 components: sampling days by week and recruitment rate by age group and quarter of year. The first component is 7 divided by the number of days per week of enrollment, usually 4. For the second component, the quarterly enrollment rate for each of 3 age strata was calculated. The first component was divided by the second component to give the final weight, which was multiplied by the age-specific numbers of enrolled children.

Rates were calculated by dividing the weighted (NVSN) or unweighted (EIP) number of influenza hospitalizations by the population estimates for Davison County obtained from the 2000 US Census. We assumed that the population of children <6 months of age was half the number of children <1 year of age. Sensitivities of each surveillance system were calculated by dividing the rates generated by each of these systems by the rate generated through the capture-recapture estimates. Analyses were performed with Stata version 8.2 software (Stata Corporation, College Station, TX).

Results

During the 2003–2004 nine-week influenza season in Davidson County, NVSN identified 274 eligible children admitted with acute respiratory infections or fever and enrolled 250 (91%), of whom 29 (11.6%) had influenza. Nonenrolled children included 18 whose parents were not available or refused to give consent, 3 who had non-English–speaking parents and no translator was available, 2 who were discharged before parents could be interviewed, and 1 who was missed. EIP identified 34 cases meeting its selection criteria through a systematic review of laboratory and medical records. The total number of influenza-associated hospitalizations among Davidson County residents <5 years of age detected by the 2 surveillance systems was 52, 29 for NVSN with surveillance 4 days per week and 34 for EIP with surveillance 7 days per week. Eleven children were identified in both systems (matched cases). The capture-recapture analysis estimated 38 cases missed by both systems, yielding 90 (95% CI 67–145) influenza hospitalizations of children <5 years of age. Among children identified through NVSN, 3% were admitted to an ICU compared with 6% of children identified through the EIP system.

The capture-recapture estimated hospitalization rate was 2.4 (95% CI 1.8–3.8) per 1,000 children <5 years of age (Table 1). Children <6 months of age had the highest hospitalization rate, 9.1 hospitalizations per 1,000 children, followed by children 6–23 months of age with 3.0 hospitalizations per 1,000 children. After weighting for sampling days and nonenrollment, the overall NVSN estimated hospitalization rate for children <5 years of age was 1.7 per 1,000, yielding an overall sensitivity of 73% compared with capture-recapture estimates (Table 2). EIP, which could only detect a clinical laboratory test with a positive result for influenza, had an estimated hospitalization rate of 0.9 per 1,000 children, yielding a sensitivity of 38% when compared with the capture-recapture estimation. (Figure 2)

Both surveillance systems sought to estimate the total number of influenza hospitalizations in county residents <5 years of age, but selection criteria differed. Children that were missed by 1 system and detected by the other were identified (Table 3). For NVSN, 16 (70%) of 23 missed case-patients were identified during nonsurveillance days and therefore not enrolled. In addition, 1 child's parent refused enrollment. By design, NVSN rates were adjusted for missed days of surveillance and nonenrolled cases (*14*). However, 6 patients hospitalized on surveillance days were not included in the NVSN rate estimation. Three of these 6

Table 1. Estimated number of children <5 years of age hospitalized with laboratory-confirmed influenza, hospitalization rates, and rate ratios, Davidson County, Tennessee, 2003–2004 influenza season*

Age, mo	Influenza hospitalizations (95% CI)	Population	Hospitalizations per 1,000 (95% CI)	Rate ratio (95% CI)
<6	37 (27–59)	4,056	9.1 (6.7–14.5)	11.1 (6.1–20.7)
6–23	35 (27–57)	11,825	3.0 (2.3–4.8)	3.6 (1.9–6.7)
24–59	18 (13–29)	21,932	0.8 (0.6–1.3)	Referent
Total	90 (67–145)	37,813	2.4 (1.8–3.8)	

*Capture-recapture estimation. Age distribution derived from the New Vaccine Surveillance Network. CI, confidence interval.

children had been hospitalized in the previous 4 days and were not enrolled because they met NVSN exclusion criteria. On admission, 1 child was enrolled and tested negative for influenza by viral culture and PCR. Exclusion of these 4 cases from the capture-recapture analyses resulted in an estimated rate of 2.1 per 1,000 community-acquired influenza hospitalizations, and an NVSN sensitivity of 84%. Two additional children, 1 who had diarrhea and thus did not meet inclusion criteria, and another who met selection criteria but was missed, were not enrolled.

For EIP, 12 (67%) of 18 patients identified only through NVSN were not enrolled because no influenza test had been ordered by their physician. In addition, 5 patients were tested with influenza rapid tests but negative results were obtained. Only 1 child whose chart indicated a positive influenza rapid test result was not identified by EIP surveillance. We repeated the capture-recapture analysis that included only children who had a clinical laboratory test for influenza. This analysis resulted in an influenza hospitalization rate of 1.4 per 1,000 children <5 years of age. The sensitivity of the EIP for detecting influenza was 64% among children who had a rapid test performed.

Influenza viral culture and RT-PCR were performed on cultures from all children enrolled by NVSN. The diagnosis was made by culture alone in 7%, RT-PCR alone in 21%, and by both in 72%. All patients detected by EIP had a positive result in a commercially available rapid test. The most common test (59%) was Directigen Flu A + B (Becton Dickinson Diagnostic Systems, Sparks, MD, USA), a membrane-based enzyme immunoassay.

Discussion

With fluctuating vaccine supplies, variable onset and severity of influenza seasons each year, and new recommendations for use of influenza vaccine in children, an accurate, informative influenza surveillance system is greatly needed. During the 2003–2004 influenza season, analysis of data from 2 independent surveillance systems, both of which included children <5 years of age, provided better estimates of hospitalization rates since it accounted for those cases undetected by each system.

NVSN attempted to enroll all potential influenza admissions on surveillance days and used the most sensitive and specific diagnostic tests to detect influenza (*18*). Reliance on viral culture alone for influenza diagnosis would have missed 21% of NVSN cases, whereas use of RT-PCR would have missed only 7%. The combination of these techniques increased the detection of influenza by the NVSN. In addition, nonsurveillance days and children whose parents refused enrollment were taken into account in NVSN rate calculations. With intense surveillance, NVSN detected 73% of influenza hospitalizations estimated by the capture-recapture analysis. Exclusion of the 4 possible nosocomial cases increased the sensitivity of NVSN to 84%. NVSN selection criteria were established to specifically exclude nosocomial cases, including children discharged within 4 days of readmission. One child was enrolled by NVSN and tested negative for influenza virus on admission but had a clinical laboratory test result indicating influenza after >1 week of hospitalization. Three other children were excluded by NVSN criteria because of a recent hospitalization. However, with available information, whether these were nosocomial infections could not be determined. Based on results of the capture-recapture analysis, NVSN modified its methodology in subsequent years to include children recently hospitalized.

The EIP surveillance system sought to find all hospitalized children with positive clinical laboratory test results for influenza. One limitation of the EIP was that influenza ascertainment relied on a diagnostic test ordered by the physician. Another limitation was the sensitivity of the

Table 2. Influenza hospitalization rates per 1,000 children <5 years of age and sensitivity of system compared to capture-recapture estimates, Davidson County, Tennessee, 2003–2004 influenza season*

	Hospitalization rates (%) per 1,000 children		Sensitivity (%) compared to capture-recapture estimates	
Age, mo	NVSN†	EIP	NVSN	EIP
<6	6.66	3.45	72.97	37.84
6–23	2.20	1.18	74.29	40.00
24–59	0.59	0.27	72.22	33.33
Total	1.75	0.90	73.33	37.78

*NVSN, New Vaccine Surveillance Network; EIP, Emerging Infections Program.
†Estimation corrected for nonsurveillance days and nonenrolled children.

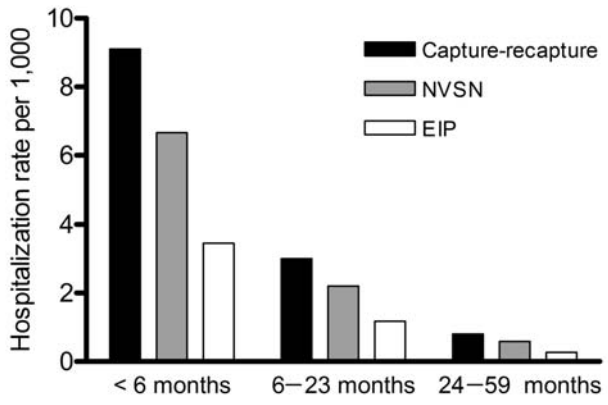

Figure 2. Influenza hospitalization rates in children <5 years of age by capture-recapture estimates and surveillance systems, Davidson County, Tennessee, 2003–2004 influenza season. NVSN, New Vaccine Surveillance Network; EIP, Emerging Infections Program.

rapid influenza detection tests. When tests were not ordered or yielded false-negative results, influenza cases were undetected. EIP surveillance was cheaper and logistically simpler to implement than NSVN. Since EIP was considered a public health response program in Tennessee, it did not require parental informed consent. However, EIP will underestimate the impact unless combined with additional information on the proportion of patients with true cases who are tested and the sensitivity of the diagnostic tests used. During its first year of influenza surveillance in Davidson County, EIP missed only 1 patient who could have potentially been detected. However, because the rapid influenza antigen test, the only clinical laboratory influenza test used in these patients, was less sensitive than RT-PCR plus viral culture, the estimated sensitivity of EIP for children who actually had clinical laboratory tests performed was 64%. When compared with viral culture, these tests have a sensitivity ranging from 44% to 95% and a specificity ranging from 76% to 100% (*27–30*). Although the ability of EIP to detect influenza cases was dependent on these test characteristics, the primary reason for EIP's underestimation of rates was that diagnostic tests for influenza were not ordered for most children admitted with influenza. The capture-recapture analysis indicated that only 38% of children <5 years of age hospitalized with influenza were correctly identified by routine diagnostic tests. Thus, not detecting influenza during hospitalization resulted not only in underestimating the impact of influenza, but also in providing limited opportunity for appropriate antiviral therapy.

Table 3. Nonenrolled influenza patients by surveillance system, Davidson County, Tennessee, 2003–2004 influenza season*

Case-patients not enrolled by NVSN	No.
Hospitalized on nonsurveillance days	16
Refused enrollment	1
Hospitalized in past 4 days, excluded	3
Enrolled, tested negative for influenza at admission	1
Hospitalized with nonrespiratory symptoms, excluded	1
Missed	1
Total	23
Case-patients not enrolled by EIP	
No influenza tests ordered	12
Rapid test for influenza done, negative result	5
Missed	1
Total	18

*NVSN, New Vaccine Surveillance Network; EIP, Emerging Infections Program.

Capture-recapture methods emerged as an adaptation of techniques used by wildlife researchers to obtain better counts of difficult-to-enumerate wild animals. The simplest technique uses 2 samples or lists. Using the number of individuals caught in each sample (captures) and the number of subjects from the first sample that were captured again by the second sample (recaptures), one can estimate the number of subjects not caught in either sample, thus providing an estimate of the total population size (*31–34*). The estimation directly accounts for different capture probabilities of each sample, and allows one to obtain estimates using 1 source that operated 4 days a week (NVSN) and the other that operated continuously (EIP).

Since being identified in 1 system did not influence the possibility of identification in the other system, the independence of the 2 systems was assumed. The independence assumption could have been violated if some factor, such as severity of influenza illness or viral load, varied among subjects and the likelihood of detection increased in both systems with increasing severity or viral load. In this case, the Peterson method would underestimate the true population size. In addition, both systems would likely miss children with very low or no influenza viral loads, such as those admitted late in the course of illness. This would also underestimate the true rates.

No significant migration occurred in Davidson County during the study, and the study population was restricted to county residents and assumed to be closed. This study was conducted during a single influenza season and there were relatively small numbers of cases identified, which precluded detailed subgroup analyses. However, the final estimation of influenza hospitalization rates was consistent with previous reports of the 2003–2004 influenza season and with previous research indicating that children <24 months of age have hospitalization rates similar to those of persons ≥65 years of age (*11,12,20,35*). This estimation also highlights the great impact of influenza, particularly in children <6 months of age during a moderately severe influenza season. Current vaccines are poorly immunogenic in this age group and have not been approved for

these children. Thus, vaccination of household contacts and out-of-home caregivers of children <6 months of age is recommended. Additional influenza vaccination of children 6–23 months of age has also been recommended to limit their exposure (*35*,*36*). As immunization rates in families of young children increase and routine vaccination for children 6–23 months of age is implemented, surveillance systems must be in place to effectively measure the impact of these preventive strategies.

Combined NVSN and EIP systems analyzed with the capture-recapture approach appear well suited to this important task. EIP is a simpler and cheaper system for identifying children with influenza. Although EIP could estimate rates more accurately by adjusting for known sensitivities and specificities of clinical diagnostic tests, without information on the frequency of diagnostic testing, it would be impossible to determine and adjust for the proportion of influenza this system captures. Thus, the degree of underascertainment would be unknown. In addition, such diagnostic testing will likely change over time, making year-to-year comparisons of disease impact difficult. NVSN attempted to estimate the true impact of influenza hospitalizations by testing all children with specific admission criteria, adjusting for nonenrollment and nonsurveillance days, and providing an unbiased sample of influenza-positive children for further analyses such as vaccine effectiveness estimates. However, this system also underestimated the total influenza impact. The combined systems gave the best estimate of disease impact.

Currently, no population-based surveillance systems are available to monitor the influenza vaccine program in adults. Using a combination of 2 systems similar to NVSN and EIP could be a model for surveillance of influenza in adults. The more expensive and labor-intensive NVSN-type surveillance could be conducted at representative hospitals in a geographic area for limited periods during the influenza season (e.g., 1 day/week at each hospital). The EIP-type surveillance system could attempt to identify all persons admitted with influenza identified through routine testing. Capture-recapture methods could be used to more accurately estimate serious influenza impact. Comparison of patients could determine whether those identified through cheaper EIP methods were representative of all patients with respect to important characteristics such as influenza vaccination status and severity of disease. Capture-recapture techniques should be considered as methods to best use limited resources for essential surveillance activities.

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Dr Grijalva is a research fellow in the Department of Preventive Medicine, Vanderbilt University Medical Center. His primary research interest is vaccine-preventable diseases.

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Address for correspondence: Marie R. Griffin, A-1110 Medical Center North, Preventive Medicine Department, Vanderbilt University Medical Center, Nashville, TN 37232-2637, USA; fax: 615-343-8722; email: marie.griffin@vanderbilt.edu

Real-time Estimates in Early Detection of SARS

Simon Cauchemez,*† Pierre-Yves Boëlle,*†‡ Christl A. Donnelly,§ Neil M Ferguson,§ Guy Thomas,*†‡ Gabriel M Leung,¶ Anthony J Hedley,¶ Roy M Anderson,§ and Alain-Jacques Valleron*†‡

We propose a Bayesian statistical framework for estimating the reproduction number *R* early in an epidemic. This method allows for the yet-unrecorded secondary cases if the estimate is obtained before the epidemic has ended. We applied our approach to the severe acute respiratory syndrome (SARS) epidemic that started in February 2003 in Hong Kong. Temporal patterns of *R* estimated after 5, 10, and 20 days were similar. Ninety-five percent credible intervals narrowed when more data were available but stabilized after 10 days. Using simulation studies of SARS-like outbreaks, we have shown that the method may be used for early monitoring of the effect of control measures.

The reproduction number *R* of an epidemic (the mean number of secondary cases infected by a single infectious case) is a key parameter for the analysis of infectious diseases because it summarizes the potential transmissibility of the disease and indicates whether an epidemic is under control ($R<1$). Up to now, this parameter has only been estimated retrospectively for periods from which all secondary cases had been detected. In terms of policy development and evaluation during the epidemic, obtaining estimates of the temporal trends in the reproduction number relating to as recent a time as possible would be critical.

If all incident cases could be traced to their index cases, estimating the reproduction number would simply be a matter of counting secondary cases. However, if tracing information is incomplete or ambiguous, modeling or statistical approaches are required. For example, a mathematical model for disease transmission fitted to available data can provide estimates of *R* (*1*). An approach requiring fewer assumptions has been proposed by Wallinga and Teunis (*2*), in which the distribution of the generation interval of the disease and the epidemic curve are directly analyzed and suffice to provide estimates. For an ongoing epidemic, this method could be used to estimate the number of secondary cases infected by a primary case-patient, but only for periods from which all secondary cases would have been detected. For severe acute respiratory syndrome (SARS), the required lag would be on the order of 15 days (95th percentile of the distribution of the generation interval described by Lipsitch et al.) (*3*).

In this report, we show how to estimate the reproduction number in an ongoing epidemic, which will account for yet unobserved secondary cases. The method is applied to data from the 2003 SARS outbreak in Hong Kong (*4*). Using simulated data, we demonstrate how the method may be used for early detection of the effect of control measures.

Materials and Methods

Statistical Framework

We propose a Bayesian statistical framework for real-time inference on the temporal pattern of the reproduction number of an epidemic. Here, the reproduction number R_t for day t will be defined as the mean number of secondary cases infected by a case with symptom onset at day t. Denoting n_t as the number of cases with symptom onset at day t and X_t as the number of secondary cases they infected, the reproduction number R_t is the ratio X_t/n_t, defined for $n_t>0$.

Assume that we would like to compute the daily values R_t from day 0 to present day T, before the epidemic has

*Institut National de la Santé et de la Recherche Médicale, Paris, France; †Université Pierre et Marie Curie, Paris, France; ‡Assistance Publique–Hôpitaux de Paris, Paris, France; §Imperial College, London, United Kingdom; and ¶University of Hong Kong, Hong Kong Special Administrative Region, People's Republic of China

ended. Although daily incident case counts can be known up to day T, provided no delay in reporting occurs, the corresponding counts of secondary cases X_t cannot. Secondary case-patients infected before day T, whose illness had a long incubation time, may have clinical onset only after day T. Furthermore, since the exact chain of transmission is seldom observed in practice, attributing secondary cases to previous cases is difficult. Focusing on these 2 issues, we show that the daily counts of symptom onset available until day T are sufficient to estimate R_t.

A 3-step construct is necessary. We first predict the eventual number of late secondary cases (as yet unobserved), for cases reported at day t, assuming the number of early secondary cases (reported before day T) is known. The method described by Wallinga and Teunis (*2*) is then used to estimate the number of early secondary cases from the daily counts of symptom onsets. These 2 steps are finally combined and yield an estimate of the predictive distribution of R_t. Technical details are given in the online Appendix (available from http://www.cdc.gov/ncidod/EID/vol12no01/05-0593_app.htm). The estimation procedure depends on 3 assumptions: 1) ascertainment of patients whose symptoms appear before day T is complete, 2) transmission events are independent, and 3) the generation interval, the time from symptom onset in a primary case to symptom onset in a secondary case, has a known frequency distribution.

Data from Hong Kong

The method was retrospectively used to analyze the SARS outbreak in Hong Kong. The data consisted of the dates of symptom onset of the 1,755 case-patients who were detected in Hong Kong in 2003 (*4*).

Simulated Data

Using simulations, we explored the ability of the method to quickly detect the effect of control measures. Five hundred epidemics were simulated with the following characteristics. During the first 20 days of the epidemics, the theoretical reproduction number was 3. Control measures were implemented at day 20. In a first scenario, control measures were completely effective (no transmission occurred after day 20). In a second scenario, the theoretical reproduction number after control measures were implemented was 0.7. Details on the simulations are available from the corresponding author.

In a simulation study, the bias and precision of the real-time estimator were investigated in situations in which the theoretical reproduction number remained constant with time. We also evaluated the effect of the length of the generation interval on the results. Detailed information can be obtained from the corresponding author.

Results

Application to Hong Kong SARS Data

Figure 1A shows the dates of symptom onset of the 1,755 SARS patients detected in Hong Kong in 2003. Figure 1B–F shows the expectation and 95% credible intervals of the predictive distribution of R_t based on data available at the end of the epidemic and after a lag of 2, 5, 10, and 20 days.

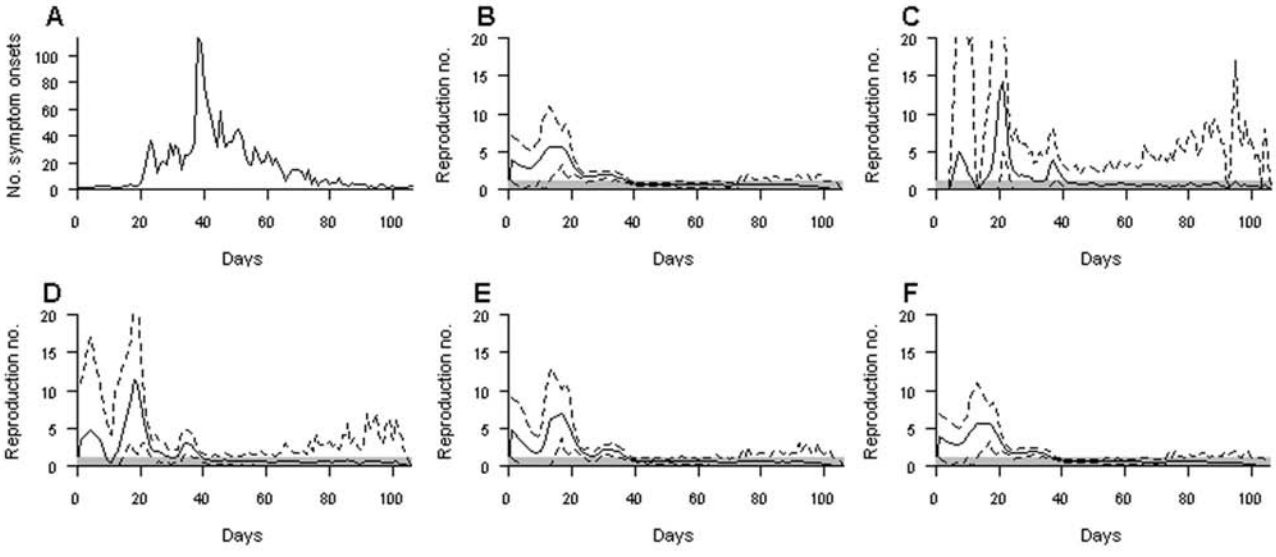

Figure 1. Application of real-time estimation to the severe acute respiratory syndrome outbreak in Hong Kong. A) Data. B–F) Expectation (solid lines) and 95% credible intervals (dashed lines) of the real-time estimator of R_t were calculated at the end of the epidemic (B) and after a lag of 2 (C), 5 (D), 10 (E), and 20 (F) days. The gray zones indicate that R is ≤1.

After a lag of 2 days, the 95% credible intervals were wide and displayed an undesirable feature: they sharply decreased to 0 as soon as no cases had been observed for 2 consecutive days (Figure 1C; note especially days 1–4 and 13). After a 5-day lag, this undesirable feature had vanished (Figure 1D).

With lags ≥5 days, the trends of expected values were relatively similar, with a peak around day 20, a decreasing trend after this date, and the expectation of R_t decreasing to <1 around day 40. These observations suggest that after a lag of only 5 days, the temporal trends in the expectation of R_t are well captured. For a lag of 5 days, the credible interval of R_t was wide when <20 cases were detected (periods $0 \leq t \leq 20$ and $t \geq 63$), but was relatively narrow when more cases were detected (period $21 \leq t \leq 62$). As expected, the width of the credible interval narrowed as the lag increased and more complete data were available. The expectations and credible intervals were very similar for lags of 10 and 20 days, 67.8th and 99.7th percentiles, respectively, of the distribution of the SARS generation interval described by Lipsitch et al. (*3*). No difference was detected between retrospective and 20-day estimates.

Detecting the Effect of Control Measures

In Figure 2, the method is used to estimate the impact of control measures implemented on day 20 in the simulated datasets with completely effective or limited control measures. The curves show the temporal pattern of R_t based on an average over the 500 simulated datasets as a function of T. Even when control measures are completely effective, based on data available up to day 21, the average expectation of R_{20} is ≈3. Based on data available up to day 25, a downward trend is apparent, whereas based on data available up to day 29, the average expectation of R_t is <1 from t = 27 days. Based on data available up to day 40 (20 days after the implementation of the control measures), the estimates indicate that the threshold value 1 is crossed at day 22, which is 2 days after control measures were implemented. With limited control measures, the observed changes are qualitatively the same, although slightly more time is required for R_t estimates to decrease to <1.

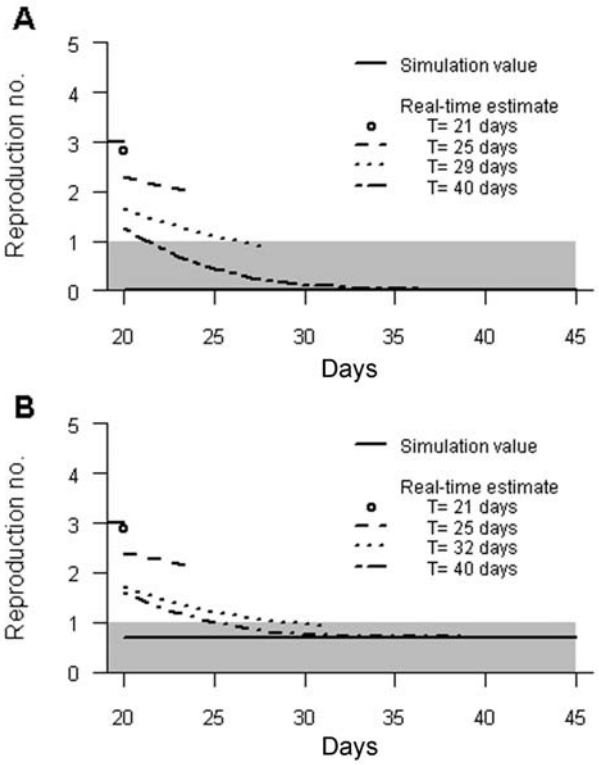

Figure 2. Average expectation of the temporal pattern of R_t after implementation of control measures according to the day T of the last observation. A) Completely effective control measures. B) Limited control measures. Simulation values of R are also given: before day 20, R = 3; after day 20, R = 0 (A) and R = 0.7 (B). The gray zone indicates that R is ≤1. Information that the average expectation of R has passed <1 was obtained 9 (A) and 12 (B) days after control measures were implemented.

Discussion

Our statistical framework provided real-time estimates of the reproduction number of an epidemic, and thus quickly showed the impact of control measures. In simulations of SARS-like diseases, the derived estimator detected the decrease of R_t only 5 days after control measures were implemented. Furthermore, the average estimate had crossed the threshold value of 1 only 9 days after control measures were implemented.

In theory, the method could be applied to communicable diseases with the following characteristics: 1) no asymptomatic cases; 2) no underreporting; 3) knowledge of the generation interval. The list of communicable diseases that could be monitored is therefore relatively large, although it does not include diseases such as influenza, for which the proportion of asymptomatic or unreported cases may be large. In practice, the delay until estimates of the reproduction number become reliable will depend critically on the generation interval distribution. For SARS, when the reproduction number was constant over time, our real-time estimates were almost unbiased after only 1 day. With the original estimator of Wallinga and Teunis (*2*), which is not intended for real-time estimation, downward bias would be a concern for at least 2 weeks after observation. However, real-time estimates obtained for recent days displayed wide 95% credible intervals and zero-width intervals when no cases had been observed or reported for a few days. Here, owing to the relatively short generation interval of SARS (mean 8.4 days) (*3*), reliable estimates were obtained after only 5 days, albeit with wide credible intervals, and they were consolidated after 10 days. These

lags corresponded to the 20th and 70th percentiles of the SARS generation interval (*3*). When the generation interval doubled, the time delay required to detect the effect of control measures implementation or to consolidate estimates roughly doubled.

We assumed that the distribution of the generation interval was known and remained unchanged during the course of the outbreak. In practice, however, this distribution is derived from a subset of traced cases. If the subset is small, e.g., the case at the beginning of an emerging disease outbreak, uncertainty will be large. Furthermore, the generation interval may decrease during the course of the outbreak because of quicker interventions, leading to possible bias in the estimates of R (*2*). Further developments of the method could take these issues into consideration. For example, one could use information on traced cases as it accrues to sequentially estimate the generation interval. Depending on how cases are traced during the epidemic, changes in the generation interval could also be monitored.

The approach smoothed the temporal pattern of the reproduction number, leading to overestimation of R in the week after control measures were implemented. We are trying to find a correction factor for this bias in ongoing research.

The method has a natural real-time implementation in which 1) a first estimate of the reproduction number is available after a lag that depends on the generation interval, and 2) while the epidemic goes on, the estimate is consolidated, and its credible interval narrows. Incorporation of such a statistical estimation framework into real-time surveillance of future infectious disease outbreaks would enhance the ability of epidemiologists to provide timely advice to public health policymakers.

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Mr Cauchemez is a doctoral student at University Pierre et Marie Curie, Paris, France. He develops statistical methods to analyze transmission of infectious diseases using incomplete information.

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Address for correspondence: Simon Cauchemez, Department of Infectious Disease Epidemiology, St. Mary's Campus, Norfolk Place, London W2 1PG, UK; fax: 44-20-0759-43282; email: s.cauchemez@imperial.ac.uk

Influenza-associated Deaths in Tropical Singapore

Angela Chow,* Stefan Ma,* Ai Ee Ling,† and Suok Kai Chew*

We used a regression model to examine the impact of influenza on death rates in tropical Singapore for the period 1996–2003. Influenza A (H3N2) was the predominant circulating influenza virus subtype, with consistently significant and robust effect on mortality rates. Influenza was associated with an annual death rate from all causes, from underlying pneumonia and influenza, and from underlying circulatory and respiratory conditions of 14.8 (95% confidence interval 9.8-19.8), 2.9 (1.0–5.0), and 11.9 (8.3–15.7) per 100,000 person-years, respectively. These results are comparable with observations in the United States and subtropical Hong Kong. An estimated 6.5% of underlying pneumonia and influenza deaths were attributable to influenza. The proportion of influenza-associated deaths was 11.3 times higher in persons age ≥65 years than in the general population. Our findings support the need for influenza surveillance and annual influenza vaccination for at-risk populations in tropical countries.

Influenza virus infections cause excess illness and deaths in temperate countries. In the Northern and Southern Hemispheres, influenza epidemics occur nearly every winter, leading to an increase in hospitalizations and deaths. The World Health Organization (WHO) estimated that these annual epidemics result in 3 to 5 million cases of severe illness and 250,000–500,000 deaths each year around the world (*1*). In the United States, influenza is responsible for 50 million illnesses and up to 47,200 deaths annually (*2–4*).

However, little is known about the impact of influenza on death rates in tropical regions, where the effect of influenza is thought to be less (*5*). In subtropical Hong Kong, deaths from underlying pneumonia and influenza attributable to influenza were estimated to be 4.1/100,000 population per year (*6*), higher than the rate (3.1/100,000) reported in the United States (*7*).

*Ministry of Health, Singapore; and †Singapore General Hospital, Singapore

In tropical Singapore, influenza viruses circulate year round, with a bimodal increase in influenza incidence observed in April–July and November–January (*8-13*). The peaks correspond approximately to increased influenza activities in temperate countries in the Southern and Northern Hemispheres, respectively (*14,15*). Singapore is geographically located in the tropics, lying just north of the equator at latitude 1.5°N and longitude 104°E. Its climate is characterized by uniform temperatures of minimum 23°C–26°C and maximum 31°C–34°C and a relative humidity of 84% with maximum rainfall occurring in April and December (*16*). These conditions are typical for most tropical countries.

Any assessment of the true impact of influenza in the tropics must account for the more diffused seasonal pattern of influenza in the tropics and the cocirculation of other respiratory viruses. Respiratory syncytial virus (RSV) is also associated with excess deaths (*17*). Thus, the effect of this virus would have to be adjusted for. In this study, we used a regression model to examine the impact of influenza, by virus type and subtype, on deaths in a tropical country, while adjusting for potential confounding effects by other cocirculating influenza virus subtypes and RSV.

Methods

National Influenza Viral Surveillance

Influenza virus surveillance is carried out throughout the year and has been instituted in Singapore since 1973. We obtained monthly data on influenza A and B viruses and RSV from the WHO-designated National Influenza Centre in Singapore from January 1996 to December 2003. Specimens tested for influenza and RSV were obtained from pediatric inpatients at KK Women's and Children's Hospital, patients from Singapore General Hospital and other public-sector hospitals, as well as from adult outpatients with influenzalike symptoms treated at sentinel primary health clinics. Specimens were tested either with

informed consent from patients for diagnostic purposes or as part of epidemiologic surveillance provided for by the Infectious Diseases Act.

RSV was detected by immunofluorescence tests and virus isolation. Influenza viruses were identified by direct antigen detection with immunofluorescence techniques, serologic tests with complement fixation, and virus isolation. To isolate influenza viruses, respiratory specimens were added to primary cynomolgus monkey kidney tissue cultures, which were rolled at 33°C and observed daily for cytopathic effects. If no effect was observed, the HeLa tubes were passaged blind at weekly intervals, and monkey kidney tissue cultures were tested for hemadsorption with guinea pig erythrocytes. Specimens were discarded after 4 weeks if negative. Influenza virus isolates were subsequently confirmed by immunofluorescence and typed by hemagglutination-inhibition tests using strain-specific antisera provided by the WHO Collaborating Centre for Influenza at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

The National Influenza Center provided aggregated data for this study, i.e., monthly numbers of total respiratory specimens tested for influenza virus, positive influenza test results, and influenza virus isolates by subtype, as well as monthly RSV data. As the study spanned 8 years, we anticipated that positive results could be affected by changes in the number of tests performed. Therefore, we opted to use the monthly proportion of positive test results for a specific virus (with the respective monthly number of specimens tested for the specific virus as the denominator) as our indicator variable for virus activity, instead of monthly positive counts.

Mortality Data

National mortality data were obtained from the Registry of Births and Deaths. Under the Registration of Births and Deaths Act, all deaths occurring within Singapore and its territorial waters are required to be registered within 3 days of the occurrence. Each death was categorized according to the International Classification of Diseases, 9th Revision (ICD-9) codes. In this study, death records were aggregated according to month of death from January 1996 through December 2003. Three death outcomes were analyzed: underlying pneumonia and influenza (P&I) deaths (ICD-9: 480–487), underlying circulatory and respiratory (C&R) deaths (ICD-9: 390–519), and all-cause deaths (ICD-9: 000–999).

Statistical Methods

We first applied 6 negative binomial regression models (*18*) to the monthly number of deaths and monthly proportions of positive influenza virus and RSV tests, to examine the relationships between mortality and the respiratory viruses (namely, models 1–6). Details of the models are shown in the Appendix. We then used the full model (model 6) to obtain the relative risks (RR) of death (and 95% confidence interval [CI]) from influenza A, A (H3N2), A (H1N1), and B viruses, as well as RSV, for each of the 3 mortality categories (i.e., all-cause, underlying P&I, and underlying C&R deaths). We also attempted to estimate the excess number of deaths from the viruses.

Apart from accounting for possible overdispersion of the data in the models, the models also adjusted for potential confounding factors, including the number of days in each month, linear and squared term of time trend, seasonality (3–4 pairs of sine and cosine terms, allowing for 3 to 4 cycles per year to capture the main seasonal variations per year), temperature, and relative humidity. Linear and squared terms of time trend were included to capture secular trends, including population growth, changes in completeness of ICD coding, and changes in diagnostic methods. For each model, residuals were examined for discernible patterns and autocorrelation by means of residual plots and partial autocorrelation function plots. Since the unit of analysis was the calendar month, the lag effects of influenza and other covariates were not necessarily taken into account.

We estimated the influenza-associated mortality fraction by dividing the number of excess deaths (the difference between observed and expected deaths) by the number of observed deaths, when the proportion of positive influenza results was set to 0 in model 6. The 95% CI for each estimated fraction was obtained by using the bootstrap resampling method with 1,000 bootstrap resamples (*19*). The number of excess deaths attributable to influenza was then derived by multiplying the total number of deaths in each mortality category by the respective influenza-associated mortality fraction (*6*,*20*). We also derived the excess mortality rate per 100,000 person-years by dividing the number of excess deaths during the study period by the sum of the annual midyear population for the entire 8-year period. All analyses were performed by using S-Plus 6.0 Professional Release 2 software (Insightful Corporation, Seattle, WA, USA).

Results

From January 1996 to December 2003, 57,060 specimens were tested for influenza virus, and 51,370 were tested for RSV. The volume of tests performed was noticeably lower in the first 2 years and in the last year of the study (Table 1). There were 9,103 positive results for RSV and 3,829 positive results for influenza. The annual mean number of tests positive for influenza A was 5.8% (range 2.6%–9.5%) and for influenza B, 0.9% (range 0.4%–1.6%). Annually, influenza A (H3N2) was the predominant influenza virus subtype in circulation. During the study

Table 1. Annual influenza virus and respiratory syncytial virus (RSV) surveillance data, Singapore, 1996–2003

	Influenza virus						RSV	
	Influenza type*			Influenza A subtype†				
Year	No. specimens tested	Influenza A–positive test results (%)	Influenza B–positive test results (%)	No. specimens tested	A (H1N1)–positive isolates (%)	A (H3N2)–positive isolates (%)	No. specimens tested	Total positive test results (%)
1996	5,140	132 (2.6)	47 (0.9)	924	1 (0.1)	15 (1.6)	4,249	868 (20.4)
1997	5,255	208 (4.0)	39 (0.7)	1,041	9 (0.9)	17 (1.6)	4,441	902 (20.3)
1998	8,934	817 (9.1)	120 (1.3)	941	3 (0.3)	40 (4.3)	7,573	1,683 (22.2)
1999	7,548	714 (9.5)	74 (1.0)	1,001	1 (0.1)	99 (9.9)	6,915	1,004 (14.5)
2000	7,716	397 (5.1)	122 (1.6)	974	34 (3.5)	61 (6.3)	7,094	1,425 (20.1)
2001	8,171	300 (3.7)	76 (0.9)	1,023	33 (3.2)	44 (4.3)	7,445	1,415 (19.0)
2002	8,317	274 (3.3)	34 (0.4)	897	3 (0.3)	58 (6.5)	7,840	1,128 (14.4)
2003	5,979	454 (7.9)	21 (0.4)	1,130	6 (0.5)	121 (10.7)	5,813	678 (11.7)
Mean	7,133	412 (5.8)	67 (0.9)	991	11 (1.1)	57 (5.7)	6,421	1,138 (17.8)

*Respiratory specimens were tested for influenza by virus isolation, direct antigen detection, and serologic tests.
†Influenza A isolates obtained from virus isolation were subtyped by using strain-specific antisera from the Centers for Disease Control and Prevention, Atlanta, GA, USA.

period, peaks in influenza A (H3N2) were observed from December 1998 to January 1999 (the predominant circulating strain was A/Sydney/5/97), December 2000 to January 2001 (A/Moscow/10/99), December 2002 to January 2003 (A/Moscow/10/99), and October–November 2003 (A/Fujian/411/2002). Smaller peaks were noted in April–July in 1996, 1997, 1998, and 2002, and from September to November in 1999 and 2003.

During the 8-year period, an annual mean of 15,616 deaths (range 15,301–16,024) occurred in Singapore. An average of 1,798 (range 1,545–2,340) underlying P&I deaths and 8,237 (range 7,833–8,715) underlying C&R deaths occurred each year (Table 2).

The Figure shows the temporal trends for death outcomes as well as influenza virus and RSV activities. Peaks in monthly influenza A viruses corresponded very well with peaks in monthly all-cause deaths, underlying P&I deaths, and underlying C&R deaths.

We tested the Spearman rank correlations between influenza and RSV, and meteorologic variables. Influenza A positivity (Spearman correlation [r] = 0.25) was weakly correlated with relative humidity. However, temperature (r = –0.71) was highly correlated with relative humidity. The influenza A (H3N2) subtype had a high correlation with influenza A (r = 0.75) (data not shown).

The relationship between deaths and each respiratory virus (influenza A, influenza B, and RSV) was examined by using a stepwise sequential approach (Table 3), i.e., first fitting each of the viruses into separate models (models 1–3), then adjusting for 1 of the other 2 viruses (models 4, 5), and finally, adjusting for all viruses in a single model (model 6). Furthermore, potential confounding factors were adjusted for and included in each model.

Influenza A had significant and robust effects on monthly all-cause deaths (RR 1.05 for each 10% change in positive test results, without adjusting for influenza B virus, RSV, and other potential confounding factors; vs. RR 1.05, after adjusting for influenza B, RSV, and other confounding factors), underlying P&I (RR 1.12 vs. RR 1.13), and underlying C&R (1.08 vs. 1.09) deaths.

In Table 4, we used model 6 (as described in Table 3) to further explore the association between influenza A virus subtypes and the 3 death outcomes. We replaced influenza A variable with influenza A subtypes and adjusted for influenza B virus, RSV, and other confounding factors. Only influenza A (H3N2) had significant (all p values <0.001) effects on all-cause deaths (RR 1.04 for each 10% change in positive test results, 95% CI 1.02–1.05), underlying C&R deaths (1.05, 1.04–1.07), and underlying P&I deaths (1.08, 1.04–1.12).

Table 2. Annual deaths in Singapore, 1996–2003*

Year	No. underlying P&I deaths (ICD-9: 480–487)	No. underlying C&R deaths (ICD-9: 390–519)	All-cause deaths (ICD-9: 000–999)
1996	1,690	8,420	15,569
1997	1,551	8,065	15,301
1998	1,781	8,286	15,649
1999	1,640	8,169	15,513
2000	1,795	8,253	15,691
2001	1,545	7,833	15,368
2002	2,077	8,158	15,811
2003	2,340	8,715	16,024

*P&I, pneumonia and influenza; C&R, circulatory and respiratory; ICD-9, International Classification of Diseases, 9th Revision.

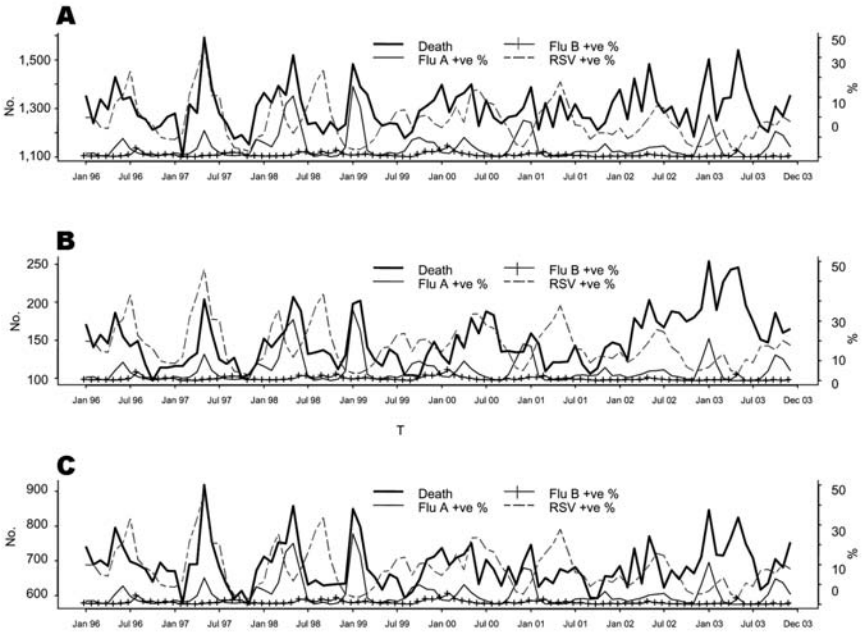

Figure. Temporal trends in the positivity of specific respiratory viruses (influenza A, influenza B, and respiratory syncytial virus [RSV]) and the number of all-cause deaths (A), underlying pneumonia and influenza (P&I) deaths (B), and underlying circulatory and respiratory (C&R) deaths (C), January 1996–December 2003; +ve%, percent positive.

Influenza B also had a significant effect on underlying C&R deaths (RR 1.01 for each 1% change in positive test results, 95% CI 1.00–1.03, p = 0.037) and all-cause deaths (1.01, 1.00–1.02, p = 0.008), but not on underlying P&I deaths (p = 0.878). RSV had no observable impact on all 3 death categories analyzed (RR range 1.00–1.01 for each 10% change in positive test results, p>0.099) (Table 4).

Next, we used the full model to quantify the excess deaths attributable to influenza throughout the year. For deaths from all causes, we estimated an annual mean of

Table 3. Adjusted risk ratios* and p values for each 10% change in positive influenza A and RSV test results, and for each 1% change in positive influenza B† virus test results, 1996–2003‡

Mortality outcome/ risk factor	Adjusted risk ratio (95% CI), p value					
	Model 1§	Model 2	Model 3	Model 4	Model 5	Model 6
All-cause deaths						
Influenza A	1.05 (1.04–1.06), 0.000	–	–	1.05 (1.04–1.06), 0.000	1.05 (1.04–1.06), 0.000	1.05 (1.04–1.06), 0.000
Influenza B	–	1.01 (1.00–1.02), 0.173	–	1.01 (1.01–1.02), 0.001	–	1.01 (1.01–1.02), 0.001
RSV	–	–	1.00 (0.99–1.00), 0.810	–	1.00 (1.00–1.01), 0.254	1.00 (1.00–1.01), 0.159
Underlying P&I deaths						
Influenza A	1.12 (1.08–1.16), 0.000	–	–	1.12 (1.08–1.16), 0.000	1.13 (1.09–1.17), 0.000	1.13 (1.09–1.17), 0.000
Influenza B	–	0.99 (0.96–1.02), 0.389	–	1.00 (0.94–1.03), 0.994	–	1.00 (0.98–1.03), 0.872
RSV	–	–	1.01 (0.99–1.02), 0.342	–	1.03 (1.00–1.02), 0.022	1.01 (1.00–1.02), 0.021
Underlying C&R deaths						
Influenza A	1.08 (1.06–1.10), 0.000	–	–	1.08 (1.07–1.10), 0.000	1.08 (1.06–1.11), 0.000	1.09 (1.07–1.11), 0.000
Influenza B	–	1.01 (0.99–1.02), 0.360	–	1.02 (1.01–1.03), 0.004	–	1.02 (1.01–1.03), 0.002
RSV	–	–	1.00 (0.99–1.01), 0.686	–	1.01 (1.00–1.01), 0.025	1.01 (1.00–1.01), 0.011

*Risk ratio estimates (95% confidence intervals) of each death category were adjusted for number of days in each month, linear and squared time trends, seasonal patterns, temperature and relative humidity; –, risk factor was not included in model.
†Each 1% change was used for influenza B because of the small range of positive influenza B test results.
‡CI, confidence interval; RSV, respiratory syncytial virus; P&I, pneumonia and influenza; C&R, circulatory and respiratory.
§Negative binomial regression models. Model 1, death outcome = influenza A + confounders; model 2, death outcome = influenza B (FluB) + confounders; model 3, death outcome = RSV + confounders; model 4, death outcome = model 1 + FluB; model 5, death outcome = model 1 +RSV; model 6, death outcome = model 4 + RSV.

Table 4. Association between influenza A virus subtypes and 3 death outcomes*

Model 6 mortality outcome	Adjusted risk ratio (95% CI), p value†			
	Influenza A (H1N1)	Influenza A (H3N2)	Influenza B	RSV
All-cause deaths	1.00 (0.96–1.04), 0.928	–	1.01 (1.00–1.02), 0.178	1.00 (0.97–1.00), 0.824
	–	1.04 (1.02–1.05), 0.000	1.01 (1.00–1.02), 0.008	1.00 (1.00–1.01), 0.484
Underlying P&I deaths	1.00 (0.88–1.13), 0.993	–	0.99 (0.96–1.02), 0.409	1.01 (0.99–1.02), 0.369
	–	1.08 (1.04–1.12), 0.000	1.00 (0.97–1.03), 0.878	1.01 (1.00–1.02), 0.099
Underlying C&R deaths	1.01 (0.95–1.08), 0.771	–	1.01 (0.99–1.02), 0.343	1.00 (0.99–1.01), 0.626
	–	1.05 (1.04–1.07), 0.000	1.01 (1.00–1.03), 0.037	1.00 (1.00–1.01), 0.166

*CI, confidence interval; RSV, respiratory syncytial virus; P&I, pneumonia and influenza; C&R, circulatory and respiratory.
†Risk ratio estimates (95% confidence intervals) of each death category were adjusted for number of days in each month, linear and squared time trends, seasonal patterns, temperature, and relative humidity; –, risk factor was not included in the model.

588 influenza-associated deaths (Table 5), representing 3.8% of total deaths. The mean annual estimates of deaths from underlying P&I and C&R associated with influenza were 116 and 475, respectively, representing 6.5% and 5.8% of such deaths.

We observed that the proportion of influenza-associated deaths was higher among the elderly. The annual influenza-associated proportion of deaths from all causes was 11.3 times higher in persons age ≥65 years (167.8/100,000 person-years) than in the general population (14.8/100,000). For influenza-associated underlying P&I deaths, the annual death rate in those ≥65 years (46.9/100,000) was 16.2 times higher than those in the general population (2.9/100,000) (Table 5).

Table 6 compares the excess deaths observed in our study with that derived from studies in a subtropical and temperate country (*6*,*7*). While we observed a smaller overall impact for all ages than that reported in Hong Kong (*6*) and the United States (*7*), we noted a higher proportion of influenza-associated deaths among the elderly in Singapore.

Discussion

To our knowledge, our findings are the first to demonstrate that influenza activity is associated with excess deaths in a tropical country. Our estimates of annual influenza-associated all-cause deaths, underlying P&I deaths, and underlying C&R deaths in Singapore were 14.8, 2.9, and 11.9 per 100,000 person-years, respectively. This finding would translate to an estimated 588 deaths (3.8% of total deaths) due to influenza annually, which is comparable to the proportion of deaths observed in subtropical Hong Kong (*6*) and in the United States (*7*), a temperate country.

Our estimate of 46.9 underlying P&I deaths per 100,000 persons age ≥65 years each year is lower than the estimate of a local study (*21*). However, that study acknowledged a possible overestimation of the incidence of influenza in the elderly. Moreover, their estimates were based on the assumption that 40% of P&I deaths were associated with influenza, which was a figure derived from external data from temperate countries. This figure far exceeds our estimate of 6.5% of underlying P&I deaths attributable to influenza. In Hong Kong (*6*) and the United States (*7*), influenza-associated deaths represented 7.4% and 9.8% of underlying P&I deaths, respectively.

In Singapore, we observed that the influenza-associated proportion of deaths was highest in persons ≥65 years. Again, this finding is consistent with those in the United States where 90% of influenza-associated deaths occurred among the elderly (*7*). In this population, we estimated an annual number of excess deaths per 100,000 population of

Table 5. Estimated influenza-associated excess deaths in Singapore, 1996–2003

Mortality outcome/age group (y)	Deaths (%) associated with influenza (95% CI)*	No. excess deaths per year (95% CI)	Excess mortality rate/100,000 person-years (95% CI)
All-cause deaths			
All ages	3.8 (2.5–5.0)	588 (396–782)	14.8 (9.8–19.8)
≥65	4.2 (2.7–5.6)	421 (273–571)	167.8 (107.0–229.5)
20–64	2.3 (0.9–3.7)	114 (42–186)	4.2 (1.6–6.8)
Underlying pneumonia and influenza deaths			
All ages	6.5 (2.2–10.5)	116 (40–196)	2.9 (1.0–5.0)
≥65	7.7 (3.5–11.7)	118 (50–189)	46.9 (20.3–74.6)
20–64	9.6 (3.0–15.7)	23 (7–39)	0.8 (0.2–1.4)
Underlying circulatory and respiratory deaths			
All ages	5.8 (4.0–7.5)	475 (324–629)	11.9 (8.3–15.7)
≥65	6.2 (4.4–8.1)	390 (270–512)	155.4 (108.8–203.0)
20–64	4.6 (2.5–6.7)	88 (47–131)	3.2 (1.7–4.8)

*CI, confidence interval.

Table 6. Annual influenza-associated deaths in Singapore, Hong Kong, and United States

Author	Country	Statistical method	Influenza-associated mortality rate/ 100,000 person-years		
			All-cause	Underlying pneumonia and influenza deaths	Underlying circulatory and respiratory deaths
Chow et al.	Singapore	Negative binomial regression model was used to estimate mortality outcomes. The model was developed by using monthly number of deaths and monthly proportion of positive influenza test results. Linear and nonlinear time trends, 3–4 pairs of seasonality variables, monthly mean temperature and relative humidity, and monthly proportion of positive respiratory syncytial virus (RSV) test results were included as covariates in the model.	All ages: 14.8 ≥65 y: 167.8	All ages: 2.9 ≥65 y: 46.9	All ages: 11.9 ≥65 y: 155.4
Wong et al. (*6*)	Hong Kong	Poisson regression model was used to estimate mortality outcomes. The model was developed by using weekly number of deaths and weekly proportion of positive influenza test results. Dummy variables for each year, 2 pairs of seasonality variables, weekly mean temperature and relative humidity, and weekly proportion of positive RSV test results were included as covariates in the model.	All ages: 16.4 ≥65 y: 136.1	All ages: 4.1 ≥65 y: 39.3	All ages: 12.4 ≥65 y: 102.0
Thompson et al. (*7*)	United States	Age-specific Poisson regression models were used to estimate mortality outcomes. Each model was developed by using weekly number of deaths for the specific age group and weekly proportion of positive influenza test results. Age-specific population size, linear and nonlinear time trends, 1 pair of seasonality variables, and weekly proportion of positive RSV test results were included as covariates in each model.	All ages: 19.6 ≥65 y: 132.5	All ages: 3.1 ≥65 y: 22.1	All ages: 13.8 ≥65 y: 98.3

167.8 of all-cause deaths, 46.9 deaths from underlying P&I, and 155.4 deaths from underlying C&R attributable to influenza.

In fact, our estimates for influenza-associated deaths in persons age ≥65 years were consistently higher than those in Hong Kong and United States, for all 3 mortality outcomes. A possible reason could be the use of influenza vaccines among vulnerable elderly is higher in the United States and Hong Kong than in Singapore. Influenza vaccination for all persons age ≥65 years is a well-established recommendation of the Advisory Committee on Immunization Practice (ACIP) in the United States (*22*). Vaccine coverage among elderly persons (≥65 years) in the United States increased from 15% to 20% before 1980 to 65% in 2001 (*23*), and the national target of 60% coverage in this population has been achieved since 1997 (*24*). In Hong Kong, the use of vaccine has been limited (*25*). However, the vaccine has been recommended for institutionalized elderly since 1997, and the Department of Health has had a program to vaccinate this population since 1998 (*26*). In Singapore, influenza vaccine use has been low, and the mean annual quantity used in 2001–2002 was only ≈20,000 doses (Ministry of Health, Singapore, unpub. data). The number of persons age ≥65 averaged ≈250,000 during that period (*27*). Even if all 20,000 vaccine doses had been given to this group of persons, vaccination coverage in the elderly would not have exceeded 8% per year.

Annual influenza vaccination for persons age ≥65 years has been recommended since September 2003 in Singapore by the National Expert Committee on Immunization. Influenza vaccine efficacy for preventing death among people ≥65 years was estimated to be 68% (*28*). In a recent study, vaccine effectiveness in those >75 years of age was found to be even greater (*29*). However, such studies have yet to be conducted in the tropics. We recommend a follow-up study to estimate the impact of vaccination on influenza-associated deaths in this age group in Singapore.

With regard to influenza subtypes, we note that most seasons in the United States were dominated by influenza A (H3N2) virus (*30*); the greatest number of influenza-associated deaths were associated with influenza A (H3N2), followed by RSV, influenza B, and influenza A (H1N1) virus (*31*). Influenza A (H3N2) virus accounted for 60% and 77% of positive influenza isolates in the United States (*7*) and Hong Kong (*6*), respectively. Our findings were similar. Influenza A (H3N2) was the predominant virus subtype during our study period and had a consistently significant impact on all 3 categories of

deaths. Although influenza B was noted to have significant effects on all-cause deaths and underlying C&R deaths, the magnitudes of RRs were relatively small (RR 1.00–1.01, for each 1% change in positive test results). In addition, influenza B virus did not have any significant and observable impact on underlying P&I deaths. We did not observe any significant impact from influenza A (H1N1) virus and RSV on all 3 outcomes.

One limitation of our study may have been that the effect of RSV could have been obscured when we analyzed data on all ages. This virus is known to predominately affect children <2 years of age (*8–10*), <5% of the population. However, this factor does not negate the main finding that influenza infections are associated with substantial disease in Singapore.

Studies suggest that global interhemispheric circulation of epidemics follows an irregular pathway with recurrent changes in the leading hemisphere (*32*). As a major travel hub with a high volume of travelers from both hemispheres, Singapore could be a sentinel for detecting changes in the circulating virus strain and contribute toward an understanding of influenza virus circulation pathways. The prevalence of influenza in Singapore illustrates the importance of improving worldwide coverage and quality of virologic and epidemiologic surveillance for influenza, as described in WHO's Global Agenda for Influenza Surveillance and Control (*33*).

Our findings have a few policy implications. First, they support the recent recommendation by the National Expert Committee on Immunization on annual influenza vaccination for elderly Singaporeans and for persons at high risk of having complications from influenza. Second, the finding that influenza infections account for substantial disease supports our continued investment in strengthening influenza surveillance in our country. Finally, the study provides justification for stockpiling antiviral drugs in our national influenza pandemic preparedness plan. An influenza pandemic can be expected to result in far higher attack and death rates (*34*,*35*) than currently observed. The extent of disease and economic impact caused by an influenza pandemic could be greatly reduced by the appropriate use of vaccines and antiviral drugs (*36*).

Conclusion

In 2003, a new infectious disease, severe acute respiratory syndrome (SARS), emerged, which caused 238 cases and 33 deaths in Singapore (*37*). The SARS outbreak galvanized public health actions in surveillance and control. Surveillance and plans for containing a resurgence of SARS remain in place, in spite of the low risk of a recurrence. Influenza, in contrast, has caused an average of 588 excess deaths in Singapore annually. Influenza continues to cause an increasing amount of disease in Singapore, particularly in our rapidly aging population. However, available strategies in influenza prevention and control have yet to be optimized, largely because the true impact of influenza has been masked by the lack of a clear seasonal pattern in the tropics. The extent of the infection has remained largely unseen.

Our study is the first to show unequivocally that influenza has a significant impact on proportion of deaths in a tropical country like Singapore. The estimated excess deaths, while less than that observed in subtropical and temperate countries, still constitutes a substantial problem. As influenza-associated deaths are largely preventable through vaccination and the judicious use of antiviral drugs, our findings can influence the public health management of this disease.

Dr Chow is a public health physician and currently oversees the Communicable Diseases Surveillance Branch at Singapore's Ministry of Health. Her research interests include infectious disease epidemiology and public health surveillance.

Appendix

We developed 6 negative binomial regression models to examine the relationships between proportion of deaths and the respiratory viruses, namely, influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) (Table 3). The models were written as follows:

Model 1

Monthly number of deaths = monthly proportion of influenza A + number of days in each month + linear time trend + squared time trend + 3–4 pairs of seasonality variables + monthly mean temperature + monthly mean relative humidity

Model 2

Monthly number of deaths = monthly proportion of influenza B + number of days in each month + linear time trend + squared time trend + 3–4 pairs of seasonality variables + monthly mean temperature + monthly mean relative humidity

Model 3

Monthly number of deaths = monthly proportion of RSV + number of days in each month + linear time trend + squared time trend + 3–4 pairs of seasonality variables + monthly mean temperature + monthly mean relative humidity

Model 4

Monthly number of deaths = monthly proportion of influenza A + monthly proportion of influenza B + number of days in each month + linear time trend + squared time trend + 3–4 pairs of seasonality variables + monthly mean temperature + monthly mean relative humidity

Model 5

Monthly number of deaths = monthly proportion of Influenza A + monthly proportion of RSV + number of days in each month + linear time trend + squared time trend + 3-4 pairs of seasonality variables + monthly mean temperature + monthly mean relative humidity

Model 6

Monthly number of deaths = monthly proportion of influenza A + monthly proportion of influenza B + monthly proportion of RSV + number of days in each month + linear time trend + squared time trend + 3-4 pairs of seasonality variables + monthly mean temperature + monthly mean relative humidity

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Address for correspondence: Angela Chow, College of Medicine Building, 16 College Rd, Singapore 169854; fax: 65-6221-5528; email: angela_chow@moh.gov.sg

Real-time Forecast of Multiphase Outbreak

Ying-Hen Hsieh* and Yuan-Sen Cheng*

We used a single equation with discrete phases to fit the daily cumulative case data from the 2003 severe acute respiratory syndrome outbreak in Toronto. This model enabled us to estimate turning points and case numbers during the 2 phases of this outbreak. The 3 estimated turning points are March 25, April 27, and May 24. The estimated case number during the first phase of the outbreak between February 23 and April 26 is 140.53 (95% confidence interval [CI] 115.88–165.17) if we use the data from February 23 to April 4; and 249 (95% CI: 246.67–251.25) at the end of the second phase on June 12 if we use the data from April 28 to June 4. The second phase can be detected by using case data just 3 days past the beginning of the phase, while the first and third turning points can be identified only ≈10 days afterwards. Our modeling procedure provides insights into ongoing outbreaks that may facilitate real-time public health responses.

Mathematical models have been used to predict the course of epidemics, albeit with mixed results (*1*). Whether and how infectious diseases are likely to spread (*2–4*) are affected by stochastic events (*5*). Once outbreaks have begun, knowing their potential severity helps public health authorities respond immediately and effectively. Much relevant information is contained in the answers to 2 questions: 1) Is the current outbreak getting better or worse? 2) How many people will be infected before the outbreak ends? Attempts to answer these questions in the early stages of an epidemic can be futile and at times misleading (*6*); nonetheless, we can address them with an appropriate mathematical model once sufficient time has elapsed (*7*). Moreover, answers can be accurate if no stochastic event occurs that could substantially alter the course of outbreaks.

We use a variation of the single-equation Richards model (*8*) to answer these key questions. Unlike models with several compartments commonly used to predict the spread of disease, the Richards model considers only the cumulative infective population size with saturation in growth as the outbreak progresses, caused by decreases in recruitment because of attempts to avoid contacts (e.g., wearing facemask) and implementation of control measures.

The basic premise of the Richards model is that the daily incidence curve consists of a single peak of high incidence, resulting in an S-shaped epidemic curve and a single turning point of the outbreak. These turning points, defined as times at which the rate of accumulation changes from increasing to decreasing or vice versa, can be easily located by finding the inflection point of the epidemic curve, the moment at which the trajectory begins to decline. This quantity has obvious epidemiologic importance, indicating either the beginning (i.e., moment of acceleration after deceleration) or end (i.e., moment of deceleration after acceleration) of a phase. The Richards model fits the single-phase severe acute respiratory syndrome (SARS) outbreaks in Hong Kong and Taiwan (*7*,*9*) well. However, in the case of the Toronto outbreak, the second wave of nosocomial infections in May caused the epidemic curve to deviate from the standard S shape. We propose an improvised version of the Richards model that fits the epidemic in Toronto and, subsequently, provide a simple procedure for real-time forecasts of outbreaks with secondary and tertiary waves.

Methods

The Richards model is logistic and is described by a single differential equation. The equation is given below, where *I(t)* is the cumulative number of infected cases at time *t* in days:

(1) $$I'(t) = rI[1-(\frac{I}{K})^a]$$

The solution is:

(2) $$I(t) = \frac{K}{\left[1+e^{-r(t-t_m)}\right]^{1/a}}$$

*National Chung Hsing University, Taichung, Taiwan

During initial stages of the outbreak, when $I(t)$ is small compared to K, the growth rate r is approximated by

$$\frac{I'(t)}{I(t)}$$

or roughly the number of cases on day t over the cumulative case number through that day. We can show mathematically that t_i is the only inflection point (or turning point denoting deceleration after acceleration) of the epidemic curve obtained from this model. Moreover, $t_m = t_i + (\ln a)/r$ is equal to the inflection point t_i when $a = 1$ and approximates t_i when a is close to 1

The model parameters are as follows: K is the carrying capacity or total case number, r is the per capita growth rate of the infected population, and a is the exponent of deviation from the standard logistic curve. Because the Richards model typically exhibits a single S-shaped curve, it is not suitable for the SARS epidemic in Canada illustrated in Figure 1.

To rectify this situation, we proposed a multistage Richards model, 1 stage for each of the S-shaped segments resulting from multiple waves of infection during this outbreak. Stages are distinguished by turning points (or inflection points), denoting acceleration after deceleration at the end of each S-shaped segment, the local minima of the corresponding incidence curves. For an n-phase epidemic outbreak, $n - 1$ local minima separate the n phases. For illustration, the incidence curve for Toronto given in Figure 2 contains 2 peaks (local maximum or turning point of the first type) and 1 valley (local minimum or turning point of second type). The multistage Richards model procedure requires 5 steps. First, fit the Richards model to cumulative cases on successive days by using a standard least-square routine. For single-phase outbreaks, parameter estimates (a, r, t_i, K) will converge as the trajectory approaches carrying capacity K, as demonstrated in the Taiwan and Hong Kong SARS outbreaks (*7*,*9*). Second, if estimated parameters remain convergent until no more new cases are detected, the outbreak has only 1 phase. However, if the estimates begin to diverge from heretofore fixed values, one knows that a turning point denoting the start of a second phase has occurred. Third, locate the turning point, t_{min}, separating 2 S-shaped phases of the epidemic as the local minimum of the incidence curve (Figure 2). This is the curve for $S'(t)$ given in the equation (1). Fourth, fit the Richards model to the cumulative case curve again, but starting from $t_{min} + 1$, the day after the start of second phase. The estimated parameters (a, r, t_i, K) will again converge as the curve approaches the carrying capacity K for the second phase. Finally, repeat steps 2–4 in the event more phases occur until the outbreak ends.

By considering successive S-shaped segments of the epidemic curve separately, one can estimate the maximum case number, K, and locate the turning points, thus providing an estimate for the cumulative number of cases during each phase.

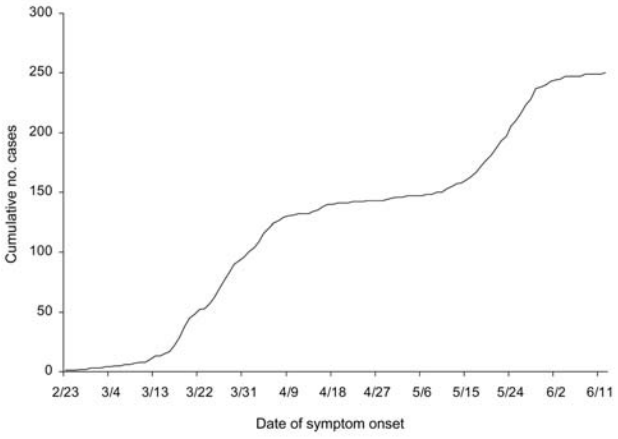

Figure 1. Cumulative severe acute respiratory syndrome cases by onset of symptoms for 250 cases in Canada February 23–June 12, 2003 (1 case had unknown onset). All except 1 of the 250 cases were in Toronto area (http://www.phac-aspc.gc.ca/sars-sras/pdf-ec/ec_ 20030808.pdf).

Results

For the phase starting February 23, we estimate parameters from data ending on various dates in Table 1. We could obtain estimates for every consecutive day after recognizing the outbreak, but we only give results for every 10 days for brevity, with the first ending on March 25. The best fitting Richards model, ending on April 26 and 28, yields the parameter values given in bold letters. The estimated value for the turning point t_i during this phase is computed from the estimates for r, a, and t_m by using equation (2). As the initial time $t = 0$ is February 23 and symptom onset occurs ≈5 days after infection (*10*), $t_i = 30.43$ gives the first inflection point around March 25 or first turning point

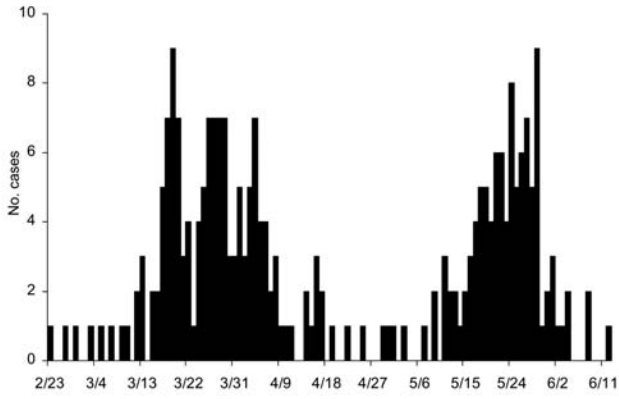

Figure 2. Severe acute respiratory syndrome incidence curve for Toronto area, February 23–June 12, 2003.

Table 1. Estimates of parameters for Richards model using cumulative case data of selected time periods in phase 1 of 2003 Toronto area SARS outbreak starting from February 23 with 95% confidence interval for the maximum case number *K**

End date	Growth rate	Exponent of deviation	Turning point	Maximum case no.
Mar 25	0.859	4.835	25.09	60.10 (54.71–65.49)
Apr 4	0.146	0.689	30.06	140.53 (115.88–165.17)
Apr 14	0.152	0.773	30.50	142.78 (137.34–148.22)
Apr 24	0.147	0.718	30.45	143.99 (141.76–146.21)
Apr 26	0.146	0.710	30.43	144.14 (142.19–146.09)
Apr 28	0.146	0.709	30.43	144.14 (142.42–145.86)
Apr 30	0.144	0.693	30.40	144.41 (142.85–145.96)
May 2	0.142	0.664	30.35	144.84 (143.40–146.29)

*SARS, severe acute respiratory syndrome.

(from acceleration to deceleration) for disease transmission in the Toronto area ≈5 days before March 20.

The number of cases during the phase ending on April 26 is 144, well approximated by our carrying capacity, K = 144.14 (95% confidence interval [CI] 142.19–146.09). Moreover, the results in Table 1 show that, using data from February 23 to April 4, or 10 days after the turning point of this phase, model fitting gives an estimate of K = 140.53 (95% CI 115.88–165.17). That is, given case data at the time of the outbreak, we could estimate the cumulative case number in the first phase accurately (Figure 3) 10 days after the turning point on March 25 and 22 days before the end of the first phase. This estimate also is the cumulative case number assuming no subsequent waves of infection.

Unfortunately, this was only the first wave in this outbreak, as indicated by estimates starting to diverge again after April 30. The last 2 rows of Table 1 suggest that the second turning point, the start of a second phase of this outbreak, occurred by April 30. Consequently, we go to step 3 in our procedure.

Here we use the incidence data starting on April 18 and continuing past April 30 to obtain a least-squares estimate of the minimum point t_{min} of the incidence curve. This choice of period ensures the minimum is contained in the time interval. Given that $t = 0$ is April 18, the least-squared estimate of the local minimum converges after May 18 and is t_{min} = 9.11 (95% CI 8.95–9.27) as shown in Table 2,

Table 2. Estimates of t_{min} using incidence curve starting on April 18

End date	Turning point	95% CI*
Apr 30	5.08	4.92–5.24
May 2	5.54	5.38–5.70
May 4	4.83	4.67–4.99
May 6	7.20	7.04–7.36
May 8	8.18	8.02–8.34
May 10	6.50	6.34–6.66
May 12	8.18	8.02–8.34
May 14	7.65	7.49–7.81
May 16	8.08	7.92–8.24
May 18	9.11	8.95–9.27
May 20	9.11	8.95–9.27

*CI, confidence interval.

along with previous estimates given every other day. This finding pinpoints the second turning point of the Toronto outbreak at April 27. Hence, April 27 separates the 2 S-shaped curves spanning the respective time periods February 23 to April 26 and April 28 to June 12, the end of the outbreak.

Again, as the data used in this article are given by onset date, which occurred after ≈5 days of incubation (*10*), April 22 is the actual second turning point that foretold the second wave of infections in Toronto. The index patient for the second phase had onset of respiratory symptoms, fever, and diarrhea on April 19 (*11*), 3 days before the turning point pinpointed by this procedure. Our result also corroborates the assessment of Health Canada, which pinpointed April 21 as the start of second phase of the outbreak in Toronto (Figure 1 in [*11*]).

Starting with the second phase of the outbreak on April 28, we again fit the cumulative case data from April 28 to the Richards model. As the case number on April 28 is 144, we use a transformation of $S(t) = S_{real}(t)$–143, where $S_{real}(t)$ is the actual data at time t, so the initial data on April 28 used here is $S(0) = 1$. We again fit the model to the cumulative data ending on various dates past May 25; the results are given in Table 3 and Figure 4. The estimates start to converge after June 4, in the last 2 rows of Table 3 in bold,

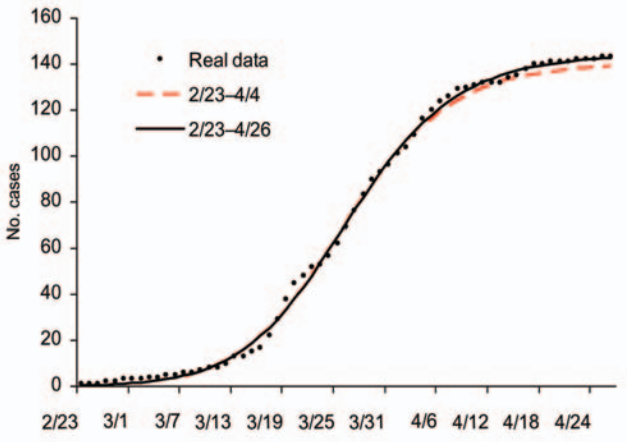

Figure 3. Epidemic curves for the first phase of severe acute respiratory syndrome outbreak in Toronto area using multistage Richards model and cases February 23–June 12, 2003.

Table 3. Estimates of parameters for Richards model using cumulative case data of selected time periods in phase 2 of 2003 Toronto area SARS outbreak starting from April 28 with 95% confidence interval for the maximum case number *K**

End date	Growth rate	Exponent of deviation	Turning point	Maximum case no.
May 25	0.557	3.866	24.59	223.37 (199.67–247.07)
May 27	0.350	2.393	25.84	244.36 (220.53–268.18)
May 29	0.236	1.554	27.36	271.28 (240.94–301.62)
May 31	0.321	2.202	26.43	252.53 (244.32–260.74)
Jun 2	**0.352**	**2.448**	**26.36**	**249.51 (245.70–253.33)**
Jun 4	**0.359**	**2.508**	**26.36**	**248.96 (246.67–251.25)**
Jun 6	**0.367**	**2.576**	**26.37**	**248.52 (246.98–250.07)**

*SARS, severe acute respiratory syndrome.

yielding an estimate for K of 248.96 (95% CI 246.67–251.25). Once again, the actual case number of 249 for the Toronto area outbreak (and 250 for Canada) is well approximated by our estimate of K. The estimated turning point t_i = 26.36 pinpoints May 24, or a turning point for SARS infections 5 days earlier on May 19. This finding further corroborates Health Canada's assertion that, among the 79 cases that resulted from exposure at the hospital where the index patient of the second phase stayed, 78 had exposures that occurred before May 23 (*11*). Note also that this estimate is obtained by using data that end just 11 days after the turning point on May 24, giving an accurate prediction of the actual cumulative case number (Figure 4).

Discussion

We show that the first turning point on March 25 could be detected 10 days after it occurred on April 4 (row 2 in Table 1). The second turning point on April 27, indicating that the epidemic escalated again, could be detected 5 days after it occurred by May 2 (last row in Table 1 shows the estimate for t_i diverging). And the third turning point on May 24 could be detected 7 days after it occurred on May 31 (row 4 in Table 3).

Our procedure fits the data well (Figure 5), allowing us to study retrospectively the significance of various events occurring at different times. Through this procedure, we can pinpoint retrospectively the 3 key turning points for the spread of disease during the 2-phase outbreak in Toronto area. The first turning point for the spread of SARS occurred on March 20 when the first wave of infections leveled off. April 22 was the second turning point, at which time persons infected by the undetected index patient for the second wave began to experience symptoms. Our findings also concur with the World Health Organization action that lifted a travel advisory issued on April 22 that limited travel to Toronto. In retrospect, the Toronto outbreak would have ended with the first wave, if not for the single undetected case and subsequent infections that occurred before April 22. Furthermore, our results also corroborate the assessment of Health Canada, which pinpointed April 21 as the start of the second phase of the outbreak in Toronto area. The third and final turning point for the infections occurred on May 19, when the spread of disease finally leveled off.

Given incidence by onset date during the outbreak, one can use our procedure to forecast the eventual severity of current phases of the outbreak by estimating the carrying capacity, K. However, accuracy depends on having the incidence data for some time past the inflection point (*7*) and no new waves of infection in the future. Both points can be aptly illustrated by the Toronto outbreak. By using data from 2/23–4/14, we can predict the 95% CI of cases in the first phase of this outbreak at 137.34–148.22, 10 days before the phase ended. Incidence data 20 days after the inflection point of the first phase (March 25) would have enabled us to project the severity of the epidemic, had there not been a second wave of infection. By performing daily fits with updated case data, one could determine if parameters were converging to reliable values for the current phase of the outbreak. Similarly, for phase 2 of the Toronto outbreak, 11 days after the final inflection point (May 24), the data from April 28 to June 4 give a good estimated 95% CI of the cumulative cases of 246.67–251.25, 8 days before onset of the last case.

These results can also be used to compute the basic reproduction number, R_0, for the Toronto outbreak. From

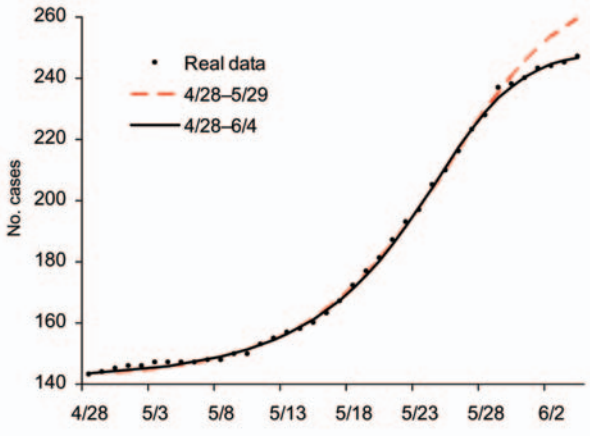

Figure 4. Epidemic curves for the second phase of severe acute respiratory syndrome outbreak in Toronto area using multistage Richards model and cases April 28–June 4, 2003.

Table 4. Comparison of basic reproduction numbers (R_0) for SARS in some affected areas in literature computed by using Richards model and T = 8.4*

Affected area	Reference	Growth rate	R_0
Singapore	9	0.12	2.7
Hong Kong	9	0.09	2.1
Taiwan	7	0.136	3.08
Toronto (phase I)	This article	0.146	3.41

*SARS, severe acute respiratory syndrome.

Table 1, r = 0.146 for the first phase. To compare with results (*9*), we also assume the duration of infectiousness T to be 8.4 days, as estimated from the time from onset of symptoms in the index patient to onset of symptoms in a secondary case-patient in Singapore (*12*) and obtain $R_0 = exp[rT] = 3.41$. The estimated r = 0.136 for Taiwan outbreak in (*7*) yields $R_0 = 3.08$. Note that, because of the shift in the cumulative number used for the model fit of the second phase, the resulting value for r cannot be used in this simple calculation. A list of basic reproduction numbers for SARS in affected areas computed in literature by using Richards model and T = 8.4 is given in Table 4 for comparison. The larger basic reproduction numbers for Toronto (phase 1) and Taiwan, as compared with Hong Kong and Singapore, may be attributable to the relatively high percentage of nosocomial infections (*13,14*).

The easily implemented procedure described can be extended to analysis of turning points and severity of multiphase epidemics while ongoing. During an outbreak such as SARS, to which available data were limited and uncertain, a simple model that requires only the most basic and perhaps only easily obtainable data under these circumstances offers our best chance to a practical solution to the understanding, prediction, and timely control of the outbreak. However, one must understand that mathematical models do not provide accurate numerical predictions and can be used to forecast only in fairly gross terms (*15*). The accuracy of predictions depends heavily also on the assumption that no stochastic events occur in the remaining days that could significantly alter the course of the current phase of an outbreak.

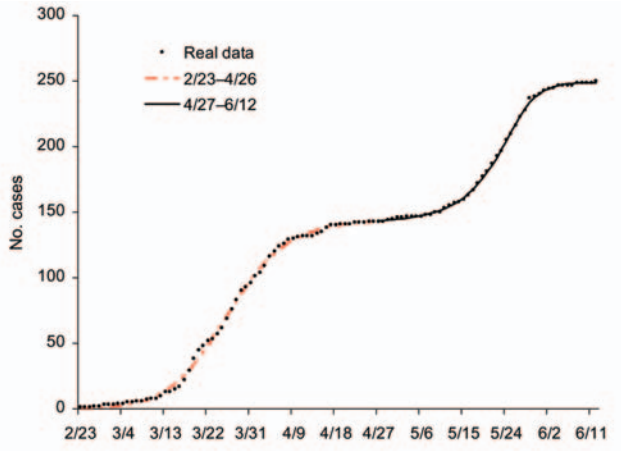

Figure 5. Epidemic curve for Toronto area severe acute respiratory syndrome outbreak of February 23–June 12, 2003, using multistage Richards model. Turning points are March 25, April 27, and May 24.

Detecting the occurrence of a second turning point or start of a second phase, as outlined in Step 2 of our procedure, is especially useful as it allows us to recognize early that an epidemic is worsening, in our case on April 30 only 3 days after the turning point on April 27 (Table 1). Though predicated on the availability and accuracy of case onset data, this procedure could be a valuable tool to public health policymakers for responding to future disease outbreaks with multiple turning points.

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Dr Hsieh is a professor of applied mathematics at National Chung Hsing University. His primary research interests are focused on mathematical and statistical modeling of infectious diseases epidemiology.

Mr Cheng received his master's degree in June of 2005 from National Chung Hsing University (Department of Applied Mathematics). His research interests are in the area of mathematic epidemiology.

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Address for correspondence: Ying-Hen Hsieh, Department of Applied Mathematics, National Chung Hsing University, 250 Kuo-Kuang Rd, Taichung, Taiwan 402; fax: 886-4-2285-3949; email: hsieh@amath.nchu.edu.tw

SARS–associated Coronavirus Replication in Cell Lines

Matthew Kaye,* Julian Druce,* Thomas Tran,* Renata Kostecki,* Doris Chibo,* Jessica Morris,* Mike Catton,* and Chris Birch*

Given the potential for laboratory-associated severe acute respiratory syndrome–associated coronavirus (SARS-CoV) infections, we must know which cell lines are susceptible to the virus. We investigated 21 cell lines routinely used for virus isolation or research. After infection with SARS-CoV, cells were observed for cytopathic effects, and quantitative real-time polymerase chain reaction was used to measure ongoing viral replication. An indirect immunofluorescence assay was also used as a confirmatory test. The study identified 10 new cell lines capable of supporting the replication of SARS-CoV and confirmed the susceptibility of 4 cell lines previously reported. This study shows that SARS-CoV can be isolated in several cell lines commonly used for diagnostic or research purposes. It also shows that SARS-CoV can achieve high titers in several cell lines, sometimes in the absence of specific cytopathic effects.

Severe acute respiratory syndrome (SARS) was first observed in 2002 when cases of a life-threatening atypical pneumonia occurred in Guangdong Province, China (*1*). A novel coronavirus (CoV), designated SARS-CoV, was quickly identified as the etiologic agent (*1*,*2*). Although the origins of the virus have not been established, evidence suggests that it is an animal virus that was recently transmitted to humans (*3*). Several wildlife species consumed as delicacies in southern China, including Himalayan masked palm civets, Chinese ferret badgers, and raccoon dogs, possess antibodies consistent with natural infection with related CoVs (*4*).

Unlike the other currently recognized human CoVs, HCoV-229E, HCoV-OC43, HCoV-NL63, and HKU1, which usually cause mild upper respiratory tract infections and occasionally pneumonia in older adults, neonates, and immunocompromised patients (*5–8*), SARS-CoV causes severe febrile lower respiratory tract illness that leads to pneumonia and acute respiratory distress (*9*,*10*). Death from progressive respiratory failure due to alveolar damage occurs in ≈10% of patients with symptomatic infection (*2*,*10*). Currently the world is free of SARS, but we cannot predict whether the virus will reemerge. The most probable sources of future infections are exposure to animal reservoirs or laboratories where SARS-CoV is manipulated for research purposes. Indeed, since the first epidemic, SARS has occurred on 3 occasions as a result of breaches in laboratory biosafety procedures (*11–13*). This finding highlights the importance of safely handling SARS-CoV, especially in diagnostic virology laboratories where virus isolation is performed and in research laboratories where infectious virus is handled.

SARS-CoV was first isolated in Vero E6 and FRhK cells injected with clinical specimens as part of early attempts to identify the etiologic agent of SARS (*10*,*14*). Simultaneously, these investigations showed that SARS-CoV could not replicate in a number of other cell lines routinely used for respiratory virus isolation. More recently, additional human and animal cell lines that support SARS-CoV replication have been identified (*15*). Given the potential for SARS-CoV infection to occur in a laboratory setting, we must be aware of cell lines in which it can replicate. Therefore, we investigated the susceptibility of a number of cell lines to SARS-CoV. These cells were derived from a variety of species and tissues and included those capable of supporting the replication of respiratory and enteric viruses.

Materials and Methods

Virus

An isolate of SARS-CoV, strain HKU 39849, was passaged on 2 occasions in Vero E6 cells to establish a high-titer stock that was used in all infectivity experiments. Because SARS-CoV is classified as a risk group level 4

*Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria, Australia

pathogen in Australia, all procedures performed with the virus, including infecting cell lines and viral lysis before RNA extraction, were carried out in a physical containment level 4 (PC4) laboratory.

Cell Lines

The cell lines investigated for their susceptibility to SARS-CoV are shown in the Table. They were chosen because they were present in our cell repository and were used either routinely or occasionally for virus isolation attempts as part of diagnostic or research projects. Confluent cells were maintained at 34°C in 25-mL flasks (Nunc, Roskilde, Denmark) containing 10 mL appropriate maintenance medium supplemented with fetal bovine serum (FBS) (Thermo Trace, Melbourne, Victoria, Australia), 100 U/mL penicillin, and 100 µg/mL streptomycin (JRH Biosciences, Lenexa, KS, USA). BGM, FRhK, HEK-293, HEL, Hep G2, L20, MA-104, pCMK, and RD-A cell lines were all maintained in modified Eagle medium (MEM) supplemented with 10% FBS. MDCK cells were maintained in MEM supplemented with 5% FBS. HeLa-T cells were maintained in basal medium Eagle supplemented with 10% FBS. COS, Huh-7, Vero, and Vero E6 cell lines were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% FBS. CV-1, Hep-2, LLC-Mk2, MEK, and RK-13 cells were maintained in 199 medium with 5% FBS, and A549 cells were maintained in RPMI 1640 medium supplemented with 10% FBS. Confluent cells were infected with SARS-CoV, which resulted in a multiplicity of infection of 1.7 (results not shown) or were mock-infected with medium only. An additional flask was also prepared in which the original inoculum was incubated under the same experimental conditions but within a cell-free environment.

On days 4, 7, and 11 after infection, cells were observed for SARS-CoV–specific cytopathic effects (CPE), supernatants were collected for virus detection and quantification by polymerase chain reaction (PCR), and the maintenance medium was replaced. Cells were tested for virus-specific antigens with an indirect immunofluorescence assay 11 days after infection with SARS-CoV if no CPE was observed (or when CPE developed that involved at least 75% of the cell monolayer). Eleven days after infection, cell lines negative for indicators of viral replication were blind-passaged twice for 7 days by adding 100 µL culture supernatant to the cells in question as well as to the highly susceptible Vero E6 cells. During these passages, cells were observed for SARS-CoV–specific CPE, and after the second passage, supernatants were collected for virus detection and quantification by PCR.

RNA Extraction

A 300-µL volume of lysis buffer containing guanidinium thiocyanate and Triton X-100 (Roche Diagnostics,

Table. Susceptibility of cells to SARS-CoV*

Cell line	Species of origin	Cell type	CPE	IDFA	Quantitative PCR Ct (days 4, 7, and 11)
Supports replication					
BGM	Monkey, buffalo green	Kidney epithelium	+	+	15, 15, 14
COS	Monkey	Derivative of CV-1	–	+	31, 33, 32
CV-1	Monkey, African green	Kidney fibroblast	+	+	14, 15, 15
FRhK	Monkey, rhesus	Fetal kidney	+	+	16, 16, 15
LLC-Mk2	Monkey, rhesus	Kidney epithelium	+	+	15, 14, 14
MA-104	Monkey, African green	Kidney epithelium	+	+	17, 15, 15
MEK	Monkey	Embryonic kidney	–	+	19, NT, 16
pCMK†	Monkey, cynomolgus	Primary kidney	+	+	20, 18, 17
Vero	Monkey, African green	Kidney epithelium	+	+	14, NT, NT
Vero E6†	Monkey, African green	Clone of Vero	+	+	14, NT, NT
HEK-293†	Human	Fetal kidney	+	+	16, 16, 17
Hep G2	Human	Liver hepatocellular carcinoma	+	+	23, 23, 20
Huh-7†	Human	Liver hepatocellular carcinoma	+	+	15, 15, 16
RK-13	Rabbit	Kidney epithelium	+	+	19, 17, 21
Does not support replication					
A549†	Human	Lung carcinoma epithelium	–	–	33, 32, 35
HEL†	Human	Diploid fetal lung fibroblast	–	–	31, 34, 38
HeLa-T	Human	Cervical epithelium	–	–	32, 33, 36
Hep-2	Human	Epithelium derived from HeLa-T	–	–	31, 32, 36
RD-A	Human	Rhabdomyosarcoma	–	–	33, 32, 35
MDCK†	Canine	Kidney epithelium	–	–	32, 37, 41
L20	Murine	Express poliovirus receptor	–	–	33, 32, 35

*SARS-CoV, severe acute respiratory syndrome–associated coronavirus; CPE, cytopathic effect; IDFA, indirect immunofluorescence assay; PCR, polymerase chain reaction; Ct, cycle threshold; NT, not tested.
†These cell lines were tested as part of another study (*14*), and the results confirmed as part of this study.

Mannheim, Germany) was added to 200 µL supernatant from cell cultures that had either been infected with SARS-CoV or were mock-infected. These samples were removed from the PC4 laboratory to a PC2 laboratory, where they underwent nucleic acid extraction with a MagNA Pure LC Total Nucleic Acid Isolation Kit with a MagNA Pure LC automated extraction robot (Roche Diagnostics). A 10-µL volume of eluate was treated for 10 min at 65°C and added to 12 mL reverse transcription master mix containing 5.2 A_{260} U/mL random hexamers (Roche Diagnostics), 0.17 µmol/L deoxynucleoside triphosphates (Roche Diagnostics), and 7.5 U AMV-RT enzyme (Promega, Madison, WI, USA). After incubation at 42°C for 30 min, then 100°C for 10 min, cDNA products were stored at 4°C until analyzed by PCR.

Quantitative Real-time PCR for SARS-CoV

Real-time PCR that amplified an 81-bp fragment of the nucleoprotein gene was used to detect and quantify SARS-CoV by reference to a cycle threshold (Ct). The assay used ABI-7000 Prism instrumentation (Applied Biosystems, Foster City, CA, USA) with primers and probes designed with the associated Primer Express software. The forward primer was SARNP-F: 5′-CCC AGA TGG TAC TTC TAT TAC CTA GGA-3′. The reverse primer was SARNP-R: 5′-CCA TAC GAT GCC TTC TTT GTT AG-3′. The probe was SARNP-P: 6FAM 5′-AAG CTT CAC TTC CCT ACG G-3′ with 3′ MGB. For real-time PCR, 5 µL template cDNA was added to ABI TaqMan Universal PCR Master Mix (Applied Biosystems) containing 0.9 µmol/L each primer and 0.2 µmol/L probe in a total volume of 45 mL. The cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, then 45 cycles of 15 s at 95°C and 1 min at 60°C. Reference to a standard curve (not shown) demonstrated that negative changes in Ct values of 3.6 represented increases in virus titer of 1.0 $\log_{10}$.

Indirect Immunofluorescence Assay

Cells were collected 11 days after infection if no CPE was observed by microscopy or on the day they developed CPE involving at least 75% of the cell monolayer. Cells were manually scraped from monolayers into 1 mL culture medium, then subjected to 50 kGy gamma radiation before being spotted onto a slide, air dried, and fixed in acetone for 10 min. Earlier testing showed that this dose of gamma radiation reduced the titer of SARS-CoV by at least 10^6 50% tissue culture infectious doses (results not shown). A 10-µL volume of diluted convalescent-phase serum from a SARS-CoV–infected patient was added to the fixed cells followed by incubation at 37°C for 30 min in a humidified chamber. The slides were washed twice with phosphate-buffered saline (PBS), dried, and each cell spot overlaid with 10 µL anti-human fluorescein isothiocyanate–conjugated secondary antibody (BioMérieux, Durham, NC, USA) for 30 min at 37°C. The slides were washed twice with PBS before they were mounted with cover slips. Virus-specific immunofluorescence was read by using an Axioskop UV microscope (Zeiss, Oberkochen, Germany). The final results for the indirect immunofluorescence assay, as shown in the Table, were based on the observations of 2 independent readers.

Results

Susceptibilities to SARS-CoV of the cell lines we investigated are shown in the Table. The results obtained on 21 lines are indicated: 14 were tested for the first time, and 7 had been previously reported by others (*15*). Of the 7 cell lines tested previously, we confirmed previous data that showed that 4 of them could support replication. Of the 14 lines tested for the first time, 10 were shown to support replication of SARS-CoV. In general, cells derived from nonhuman primate kidneys were susceptible. A human liver cell line (Hep G2) and rabbit kidney cells (RK-13) also supported replication.

SARS-CoV replication in BGM, CV-1, FRhK, LLC-Mk2, MA-104, pCMK, RK-13, and Vero cell lines produced a CPE as early as day 4 after inoculation, with evidence of high levels of virus-specific RNA established by quantitative PCR. CPE was focal, with cell rounding and a refractivity that was soon followed by cell detachment, and CPE quickly spread to involve the entire cell monolayer (Figure 1). In contrast, neither MEK nor COS cells produced a SARS-CoV–specific CPE (Figure 1), despite evidence of rapid (MEK) or limited (COS) replication, as determined by quantitative PCR (Figure 2) and indirect immunofluorescence testing (not shown). For the cell lines capable of supporting SARS-CoV replication, immunofluorescence results confirmed quantitative PCR results in all cases (Table).

Figure 2 shows the quantitative PCR results for representative cell lines for the 11 days during which isolation was attempted. The results are depicted as Ct values relative to the Ct values of a cell-free preparation. The cell-free preparation had an initial Ct value of 31, obtained when the original inoculum was seeded into a flask containing 10 mL DMEM. This input Ct increased to a Ct value of 40 by day 11 after infection. The supernatants from BGM, CV-1, MEK, Vero, and Vero E6 cell lines yielded Ct values 12–17 units lower than the initial cell-free inoculum by day 4 after infection. This number equated to titer increases 3.3–4.7 $\log_{10}$/mL above the input virus for these cells. The results for HeLa-T, Hep-2, and MDCK cells, representing cell lines that do not support SARS-CoV replication, are shown in Figure 2. In these cell lines, the Ct values at 4 days after infection were at levels similar to those of the cell-free inoculum. At later times, after succes-

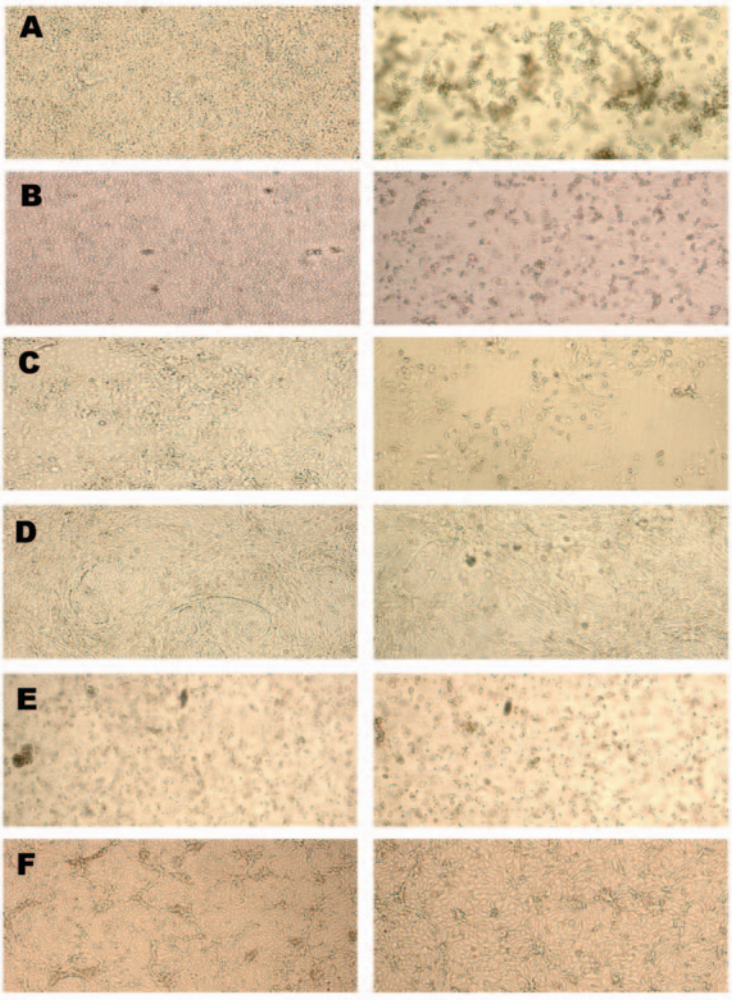

Figure 1. Cytopathology of uninfected cells (left column) and the same cells infected in vitro with severe acute respiratory syndrome–associated coronavirus (right column). A) Vero cells day 4 after infection. B) MA-104 cells day 4 after infection. C) Huh-7 cells day 11 after infection. D) pCMK cells day 11 after infection. E) COS cells day 11 after infection. F) MEK cells day 11 after infection.

sive media changes, Ct values increased in a manner similar to that of the cell-free control preparation, indicating dilution of input virus and absence of any subsequent viral replication. Blind passaging of supernatant fluid from these cell lines confirmed these results (not shown). In contrast, Ct values for COS cells did not change over the course of the experiment, which suggests that viral replication occurred at a low level, sufficient to maintain similar viral titers to those of input levels through several medium changes.

Discussion

After the SARS epidemic ended, several cases have occurred as a direct or indirect result of breaches in laboratory biosafety (*11–13*). These breaches highlight the need to safely handle virus in the laboratory, which includes knowing which cell lines may be susceptible to infection. In this study we add to the list of cells known to support replication of SARS-CoV.

Our approach to establishing susceptibility to infection was to use quantitative PCR supported by immunofluorescence testing. The quantitative PCR was used to distinguish ongoing viral production from input virus. Other groups have used alternative strategies to investigate SARS-CoV replication, including using PCR capable of amplifying subgenomic RNA molecules produced during replication (*15*). Our results show that, in laboratories where reverse-transcription PCR is not available but appropriate reagents are available, immunofluorescence testing is a simple and rapid method of assessing whether cells exposed to respiratory or enteric specimens are infected with the virus.

On the basis of this study and earlier reports (*10,14,15*), monkey kidney cell lines are particularly susceptible to SARS-CoV infection. African green, cynomolgus, and rhesus monkey kidney cell lines have all been previously shown to be susceptible. We identified for the first time that kidney cells derived from a fourth nonhuman primate species, buffalo green monkey, are productively infected with SARS-CoV, with titers that reach 4.7 $\log_{10}$/mL above input virus, similar to levels in other monkey kidney cells. We found most monkey kidney–derived cell lines, includ-

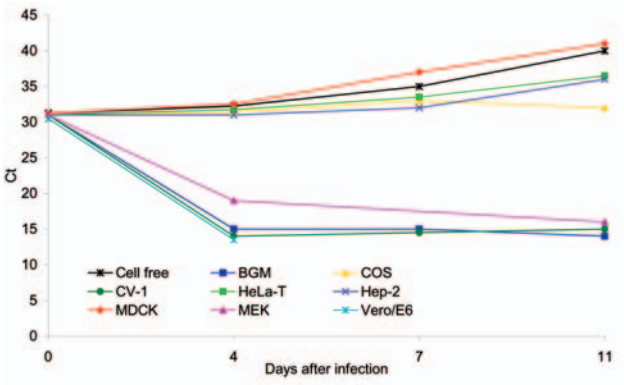

Figure 2. Cycle threshold (Ct) changes measured by real-time polymerase chain reaction versus days after infection of the indicated cell lines. The cell-free sample had an initial Ct of 31, which rose to 40 by day 11. Reductions in the Ct or flat-line Ct values (e.g., COS cells) indicate replication of the virus. Continued increases in Ct above the initial value of 31 by days 7 and 11 indicate failure to replicate.

ing BGM, CV-1, FRhK, LLC-Mk2, MA-104, pCMK, and Vero E6, supported replication of SARS-CoV, with titers 3.9–4.7 $\log_{10}$/mL above input virus titers. High titers of SARS-CoV attainable in these cell lines should be considered when using them for virus isolation purposes, and appropriate safety guidelines should be followed.

The ability of SARS-CoV to replicate efficiently in kidney-derived cell lines is not surprising given that its functional receptor, the metalloprotease angiotensin-converting enzyme 2 (ACE-2), is highly expressed in kidney tissue (*16*). This metalloprotease receptor is widely divergent from the aminopeptidase N receptor of group 1 CoVs (*16*) but is expressed in lung, heart, kidney, and gastrointestinal tissue, consistent with the pathology of SARS.

Generally, close agreement was seen between our results and those previously reported (*15*), although a difference was seen in CPE. We showed that HEK-293, Huh-7, and pCMK cells supported development of SARS-CoV–specific CPE, whereas no CPE was observed in these cell lines in an earlier study, although replication occurred (*15*). In that study, cells were observed for CPE for only 2 days after infection, whereas in the present study we observed cells for up to 11 days. In Huh-7 and pCMK cell lines, we observed that CPE often developed slowly and affected a population of cells but did not progress (Figure 1). Neither COS nor MEK cells developed SARS-CoV–specific CPE (Figure 1), despite evidence of replication by PCR and immunofluorescence. COS cells are a derivative of the African green monkey kidney fibroblast cell line CV-1, which is highly susceptible to SARS-CoV. The reason for the decreased level of virus production in related COS cells remains to be determined but may be due to a lower level surface expression of the ACE-2 receptor. Nevertheless, the results for these 2 cell lines highlight the unreliability of CPE as a measure of SARS-CoV replication.

Given that primate kidney–derived cell lines are particularly susceptible to infection with SARS-CoV and virus has been isolated from the kidney of an infected human patient (*10*), we suspect that human kidney–derived cell lines might also support SARS-CoV replication. However, until the study by Gillim-Ross et al. (*15*), no human cell lines had been shown to be productively infected by SARS-CoV. We found, in agreement with that study that, HEK-293 and Huh-7 cells were susceptible to infection with the virus. In addition, we identified a third human cell line, Hep G2, derived from a hepatocellular carcinoma, that was also susceptible to infection, although it produced lower levels of virus-specific RNA than HEK-293 and Huh-7 cells. Hep G2 and Huh-7 cell lines are used in research laboratories to study hepatitis B and C viruses, which suggests that cell lines used for research purposes need to be considered carefully for their potential to support SARS-CoV replication, and guidelines must be established to prevent simultaneous work on multiple different viruses within the same laboratory.

This study has shown that SARS-CoV can be isolated in several cell lines commonly used for diagnostic and research purposes and highlights that the virus can achieve high titers in some cell lines, sometimes in the absence of CPE. These findings are particularly relevant to laboratory scientists undertaking virus-isolation procedures on specimens collected from patients with atypical respiratory disease or in research laboratories where the possibility of simultaneously handling more than 1 virus exists.

Acknowledgment

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Mr Kaye is a medical scientist specializing in public health virology. His main interests are in the areas of technological development in diagnostic virology and physical containment level 4 practices.

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Address for correspondence: Matthew Kaye, Virology Department, Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn St, North Melbourne 3051, Victoria, Australia; fax: 61-3-9342-2666; email: matthew.kaye@mh.org.au

Ocular Vaccinia Infection in Laboratory Worker, Philadelphia, 2004

Felicia M.T. Lewis,*† Esther Chernak,* Erinn Goldman,† Yu Li,† Kevin Karem,† Inger K. Damon,† Richard Henkel,† E. Claire Newbern,* Patrina Ross,* and Caroline C. Johnson*

We report a case of ocular vaccinia infection in an unvaccinated laboratory worker. The patient was infected by a unique strain used in an experiment performed partly outside a biosafety cabinet. Vaccination should continue to be recommended, but laboratories with unvaccinated workers should also implement more stringent biosafety practices.

Vaccinia virus, the orthopoxvirus used in smallpox vaccine, is increasingly used in research laboratories, both to investigate orthopoxvirus biology and as a tool in molecular biology and immunology (*1–4*). Vaccinia can cause mild-to-moderate infection in healthy hosts and can be transmitted to their contacts (*3,5–8*). Although routine smallpox vaccination has been discontinued in the United States since 1971, vaccination is still recommended for healthcare and laboratory workers who handle nonattenuated orthopoxviruses (*6*). We document ocular vaccinia infection in an unvaccinated laboratory worker and describe the associated laboratory and epidemiologic investigation.

Case Report

An immunology graduate student born after the discontinuation of routine smallpox vaccination was working with multiple strains of vaccinia as part of her thesis research. She had voluntarily declined vaccination before beginning laboratory work with vaccinia. One morning in October 2004, she noticed the onset of itching, tearing, palpebral swelling, and conjunctival injection in her left eye. Viral conjunctivitis was diagnosed by her student health services, and over-the-counter tetrahydrozoline hydrochloride eye drops were prescribed. During the next 4 days, the eye became swollen, red, and painful; malaise, fatigue, and subjective fever also appeared. On day 5 the patient went to a private ophthalmologist, who referred her to a specialty eye hospital.

Physical examination at the eye hospital demonstrated a painful left eye with 3+ chemosis in the eyelids and conjunctiva and symblepharon at the lower pole of the eye. A 0.5-cm vesicle was noted above the left canthus (Figure 1). Left ocular range of motion, including palpebral motion, was severely limited. Keratitis was not evident. Routine laboratory values were normal. Computed tomographic scan of the orbits indicated left preseptal cellulitis without evidence of orbital cellulitis. The diagnosis of vaccinia infection was not suspected until examination at the eye hospital, when the student first mentioned her work with vaccinia. Contact precautions were then initiated, and a scraping of the vesicle above the canthus was sent to the Pennsylvania Bureau of Laboratories for vaccinia testing. The patient was started on trifluridine and bacitracin ointments, broad-spectrum systemic antimicrobial agents, and pain medication; she was admitted to the hospital.

During the next 48 hours, additional vesicles appeared on the lower conjunctiva (Figure 2), and periorbital swelling increased. Polymerase chain reaction (PCR) testing at the Pennsylvania Bureau of Laboratories showed evidence of vaccinia; results were confirmed at the Centers for Disease Control and Prevention (CDC). At this time, vaccinia immune globulin (VIG), 6,000 U/kg IV, was administered. Less than 24 hours after VIG administration, the patient's pain and swelling were substantially decreased. The patient continued to improve over the next 2 days and was discharged to her home on day 9. No long-term sequelae occurred, although recovery took several weeks.

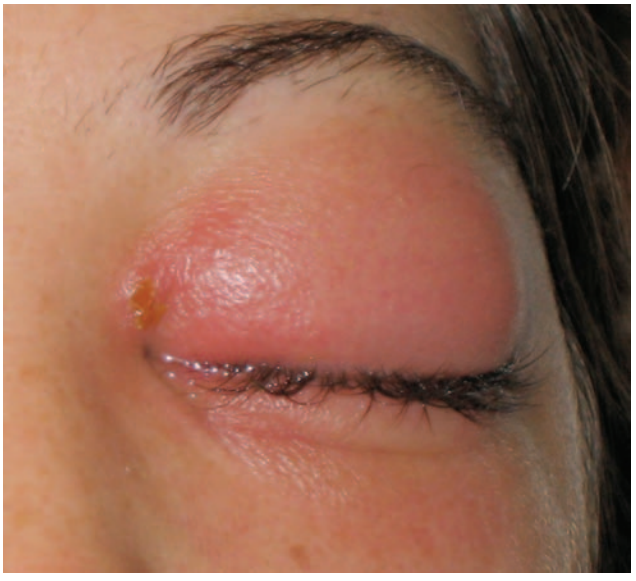

Figure 1. Patient's left eye after admission to hospital. The primary pox lesion is located at the inner canthus. Photographer: E. Claire Newbern.

*Philadelphia Department of Public Health, Philadelphia, Pennsylvania, USA; and †Centers for Disease Control and Prevention, Atlanta, Georgia, USA

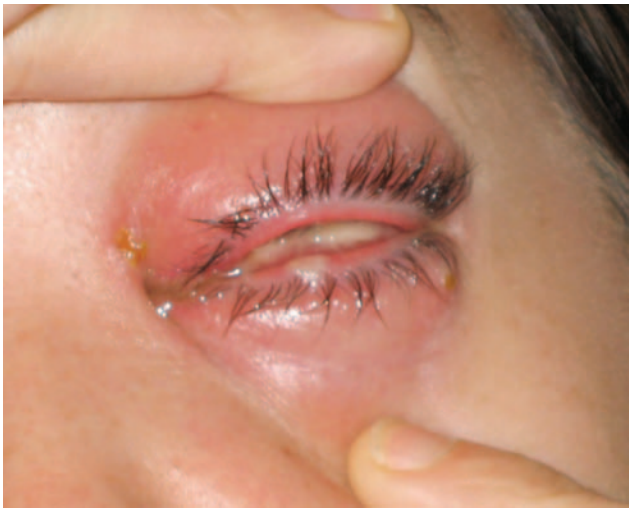

Figure 2. Satellite lesion on lower conjunctiva. Photographer: E. Claire Newbern.

A public health investigation of the patient's home and work contacts and the research laboratory was initiated. Because cutaneous lesions from vaccinia typically appear 3–5 days after inoculation (*5*), investigators postulated that the patient contracted her infection within the 7 days before symptom onset. The patient was considered infectious from the beginning of this period until hospitalization (11 days). During this time, she had 3 household contacts and 11 work contacts. Household contacts were monitored for signs of illness for 1 week. All contacts were interviewed with a standard questionnaire to ascertain the extent of their contact with the patient, vaccination status, and information about laboratory work practices, if applicable. Acute- and convalescent-phase paired serum samples were drawn at 1 and 6 weeks after exposure from all work contacts, as well as from the patient's closest home contact. Convalescent-phase serum was drawn from the patient at week 6. Paired serum samples from the patient's consenting contacts were examined for evidence of orthopoxvirus-reactive antibody by using techniques described elsewhere (*9*). Serologic results are summarized in the Table.

A laboratory inspection, which included a review of experiments performed by the patient during the week before symptom onset, was conducted, Although laboratory staff generally followed established biosafety precautions (*10*), review of laboratory practices showed several opportunities for virus exposure. Staff infrequently wore eye protection while performing experiments with vaccinia. Laboratory coat sleeves were not elasticized and did not always cover the wrist. Waste pipettes were not disinfected before removal from the biosafety cabinet. Instances occurred in which samples with low titers of live virus were removed from the biosafety cabinet, transported to other parts of the facility, and manipulated. In addition, laboratory staff routinely vortexed tubes containing live virus outside of the biosafety cabinet. Most important, no laboratory workers had been vaccinated in the past 10 years, as recommended by CDC (*6,10*).

To identify the specific infecting strain of vaccinia, the virus isolated from the patient's canthus lesion was sequenced. Briefly, a 3.7-kbp amplicon was generated and sequenced from the thymidine kinase region of the viral genome by using the following primers: TKj2r forward 5-ACGTG ATGGA TATAT TAAAG TCGAA and TKj2r reverse 5-GTTTA TCTAA CGACA CAACA TCCA.

Table. Vaccination status and serologic evidence of vaccinia immunity of patient and contacts

Patient and contact	Prior vaccination	Date of last vaccination	Anti-orthopoxvirus IgG present* (acute-phase serum, 10/04; convalescent-phase serum, 12/04)	Anti-orthopoxvirus IgM present† (acute-phase serum 10/04; convalescent-phase serum, 12/04)
Patient	No	–	–/Yes	–/Yes
Home	No	–	No/no	No/no
Worker 1	Yes, 5×‡	1994	Yes/yes	No/no
Worker 2§	Yes, as child	12/01/04	Yes/yes	No/no
Worker 3§	Yes, as child	12/01/04	Yes/yes	No/no
Worker 4§	Yes, as child	12/01/04	No/no	No/no
Worker 5	No	–	No/no	No/no
Worker 6	No	–	No/no	No/no
Worker 7	No	–	No/no	No/no
Worker 8	No	–	No/no	No/no
Worker 9	No	–	No/no	No/no
Worker 10	No	–	No/no	No/no
Worker 11	No	–	No/no	No/no

*Immunoglobulin G (IgG) optical density cutoff value (COV) = 0.214408; –, not performed.
†IgM optical density COV = 0.015763.
‡The last vaccination was ≈10 years before this incident.
§Three laboratory workers who also manipulated vaccinia were vaccinated 1 week before the convalescent-phase blood sample was drawn (workers 2, 3, and 4). All 3 had been vaccinated as children; workers 2 and 3 had orthopoxvirus-reactive IgG levels present above the COV. Worker 4 did not have IgG levels above the COV either before or after her recent vaccination.

Amplification was performed with the Expand Long Template PCR kit (Roche Molecular Biologicals, Indianapolis, IN, USA) and a Cetus Model 9700 thermocycler (Perkin-Elmer Life and Analytical Sciences, Boston, MA, USA) at 92°C × 2 min, followed by 30 cycles of 92°C × 10 s, 55°C × 30 s, and 68°C × 3 min. Purified, amplified DNA was sequenced with a CEQ 8000 Genetic Analysis System (Beckman-Coulter, Fullerton, CA, USA). Sequences were assembled using SeqMan software (DNASTAR, Inc., Madison, WI, USA).

Sequencing showed that the infecting virus was a unique form of recombinant Western Reserve vaccinia constructed in the research laboratory and routinely used by the patient; it had been last used as a control strain during a multiday experiment performed in the 5 days before the patient's symptoms began. At one point in this experiment, a 96-well plate containing small amounts of live vaccinia–infected mammalian cells was removed from the biosafety cabinet and hand-carried to another room, where the lid of the plate was removed, and the cells were examined for fluorescence. The student did not wear eye protection during this phase of the experiment; whether she wore gloves is unclear.

Conclusions

The investigation of the laboratory and examination of clinical specimens from the patient and contacts enabled investigators to pinpoint the source of infection to a single experiment. During the period when the patient could have become infected, she was the only laboratory member to use the culprit vaccinia strain, and she used it only while performing this particular experiment. Lack of seroconversion among the other staff argues against widespread environmental viral contamination in the laboratory. During the time when she could have become infected, the student had also worked with a different strain of vaccinia in titers as high as 1:1 × 10^{10} PFU/mL. However, all of the work with virus at this titer occurred in the biosafety cabinet.

Although the exact mechanism of infection could not be determined, the location of the principal lesion at the inner canthus suggests either inadvertent inoculation from hand to eye or inoculation through aerosolization of virus (*5*). Regardless, both mechanisms indicate that existing biosafety precautions in the laboratory were likely insufficient. Biosafety level 2 (BSL-2) precautions are recommended for laboratories and persons who manipulate nonattenuated strains of vaccinia virus (*10*). This recommendation assumes a priori that all such persons will be adequately vaccinated against the virus. However, this report and others of laboratory-acquired vaccinia infections demonstrate that vaccination is being waived in certain institutions (*1–3,11*). No current recommendations exist in the United States for the level of precautions to be used by unvaccinated personnel. We believe that vaccination would probably have prevented or attenuated this patient's infection and that it should continue to be recommended for laboratory workers who handle vaccinia. However, given that vaccination has risks of its own that might reduce its use (including a rate of ocular complications of 10–20/1 million immunizations) (*5,6,12,13*), biosafety recommendations for unvaccinated personnel should be specifically addressed.

Chiefly intended to protect against agents with potential for respiratory transmission, BSL-3 precautions emphasize protection from exposure to potentially infectious aerosols (*10*). CDC has previously recommended increased biosafety precautions for laboratories with unvaccinated personnel who manipulate monkeypox virus (*14*). Implementing certain BSL-3 precautions in this case, e.g., performing all manipulations of virus in the biosafety cabinet or other enclosed equipment, frequent glove changing accompanied by handwashing, and always wearing goggles or face shields when working with virus outside of a primary containment device, would have minimized the potential for human error and might have prevented this infection. Use of eye protection should be particularly stressed, as serious eye infections can occur even in previously vaccinated persons (*15*). No systematic monitoring of vaccinia infection in laboratory workers currently exists, so the full extent of the problem is unknown. Further investigation of laboratory practices involving vaccinia is warranted. At the present time, vaccination is the best way to prevent or mitigate accidental infection (*4*) and should continue to be recommended for laboratory workers handling nonattenuated strains of vaccinia. If vaccination is impossible, workers should implement more stringent biosafety practices, such as consistently using goggles and performing all manipulations of virus in the biosafety cabinet.

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Dr Lewis is Epidemic Intelligence Service officer assigned to the Philadelphia Department of Public Health. An internist and adult infectious disease specialist, her research interests include tropical medicine and the intersection of infectious disease and behavior.

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Address for correspondence: Felicia M.T. Lewis, Philadelphia Department of Public Health, 500 S Broad St, Second Floor, Philadelphia, PA 19146, USA; fax: 215-545-8362; email: bwe1@cdc.gov

Rickettsia felis Infection, Tunisia

Abir Znazen,*† Jean-Marc Rolain,* Nader Hammami,† Adnane Hammami,† Mounir Ben Jemaa,‡ and Didier Raoult*

We report, for the first time, serologic evidence of *Rickettsia felis* and *R. aeschlimannii* infections acquired in Tunisia from 1998 to 2003. We found that most patients with antibodies against both *R. conorii* and *R. typhi* had serologic evidence of *R. felis* infection.

Rickettsioses are arthropod-borne zoonoses with geographic distributions determined by the ecology of their vectors. The genus *Rickettsia* is divided into 2 groups, the spotted fever group (SFG) and the typhus group (TG), mainly based on their intracellular positions, optimal growth temperatures, gas chromatographic proportion (%), DNA content, clinical features, epidemiologic aspects, and antigenic characteristics. Recently, a new species of *Rickettsia* that infected humans, *R. felis*, was reported (*1*), and the whole genome of this species has recently been sequenced (*2*). The pathogenic role of *R. felis* in humans was demonstrated first in Texas (*1*), with subsequent reports of this "fleaborne spotted fever" confirmed in patients from Europe and South/Central America (*3*–*5*) by polymerase chain reaction (PCR), serologic tests, or both. In Tunisia, North Africa, the epidemiology of rickettsial diseases has not been documented and only 1 study concerning these diseases has been conducted. The study, conducted in 1995, confirmed that *R. conorii* and TG rickettsia were endemic in Tunisia, with estimated antibody prevalences of 9% and 3.6%, respectively (*6*). The aim of our study was to investigate the diversity of rickettsioses in Tunisia through the use of serologic assays.

The Study

Serum samples obtained from patients suspected to have clinical rickettsial infection (fever associated with an eschar or cutaneous rash) were collected from 1998 to 2003 at the Laboratory of Microbiology CHU Habib Bourguiba, Sfax, Tunisia. Acute- and convalescent-phase serum samples, when available, were stored at –80°C until they were tested in a multiple-antigen immunofluorescence assay (IFA) at the Unité des Rickettsies Marseille (*7*). Ten SFG rickettsial antigens were used: *R. conorii conorii* strain 7, *R. africae* strain ESF-5, *R. sibirica mongolitimonae* strain HA-91T, *Rickettsia aeschlimannii* strain MC16T, *R. massiliae* strain Mtu1T, *R. helvetica* strain C9P9, *R. slovaca* strain 13-B, *R. conorii israelensis* strain ISTTCDC1, *R. felis* strain URRWXCal2 ATTC VR-1525, and *R. typhi* strain Wilmington. Antigens were produced at the Unité des Rickettsies Marseille as previously reported (*8*). Immunoglobulin G (IgG) antibody titers of 1:128, seroconversion in paired serum specimens, or IgM antibody titers of 1:32 against any species were considered evidence of recent *Rickettsia* infection (*7*).

Identification at the species level was determined by Western blot (WB) and cross-adsorption assays in accordance with procedures described elsewhere (*7*,*8*). *R. conorii*, *R. aeschlimannii*, *R. felis*, and *R. typhi* isolates were suspended briefly in sterile distilled water and adjusted to 2 mg protein/mL. Twenty microliters of the preparation was electrophoresed at 100 V for 2 h through a separating gel containing 12% polyacrylamide by means of a Mini-Protean II cell apparatus (Bio-Rad, Marnes la Coquette, France). A mixture of prestained molecular mass standards (Kaleidoscope; Bio-Rad) was used to estimate the molecular masses of the separated antigens. Resolved antigens were transferred onto a 0.45-µm pore nitrocellulose membrane (Bio-Rad) that was electrophoresed for 1 h at 4°C and 100 V. The blots were blocked overnight at 4°C with 5% nonfat milk powder in Tris-buffered saline (TBS) and were washed with distilled water. Serum specimens (diluted at a ratio of 1:200 in TBS with 3% nonfat milk powder) were applied to the blots for 1 h at room temperature. After three 10-min washes in TBS with 3% nonfat milk powder, the blots were incubated for 1 h with peroxidase-conjugated goat antihuman IgG (Southern Biotechnology Associates, Birmingham, AL, USA) diluted at a ratio of 1:750 in TBS with 3% nonfat milk powder. The blots were washed 3 times in TBS, and bound conjugate was shown by incubation in a solution of 0.015% 4-chloro-1-naphthol (Sigma, Lyon, France) and 0.015% hydrogen peroxide in TBS with 16.7% methanol for 15 min. WB analysis was done both before and after cross-adsorption in accordance with procedures described elsewhere (*8*). For patients with serologic evidence of *R. felis* infection, epidemiologic and clinical data were collected from medical records.

From 1998 to 2003, 753 serum samples were collected from 638 patients in Tunisia and sent to Marseille for analysis. Paired serum specimens were available for 115 patients. Serologic evidence of recent *Rickettsia* infection was found in 86 patients. Serum samples from 63 of these patients exhibited wide cross-reactive antibodies between SFG rickettsia and either *R. felis* (45 cases) or *R. typhi* (18 cases) (Figure); 19 serum samples had cross-reactive antibodies between *R. felis* and *R. typhi*. Finally, 3 samples had cross-reactive antibodies for SFG only (except *R. felis*),

*Université de la Méditerranée, Marseille, France; †Universitaire Habib Bourguiba, Sfax, Tunisia; and ‡Universitaire Hédi Chaker, Sfax, Tunisia

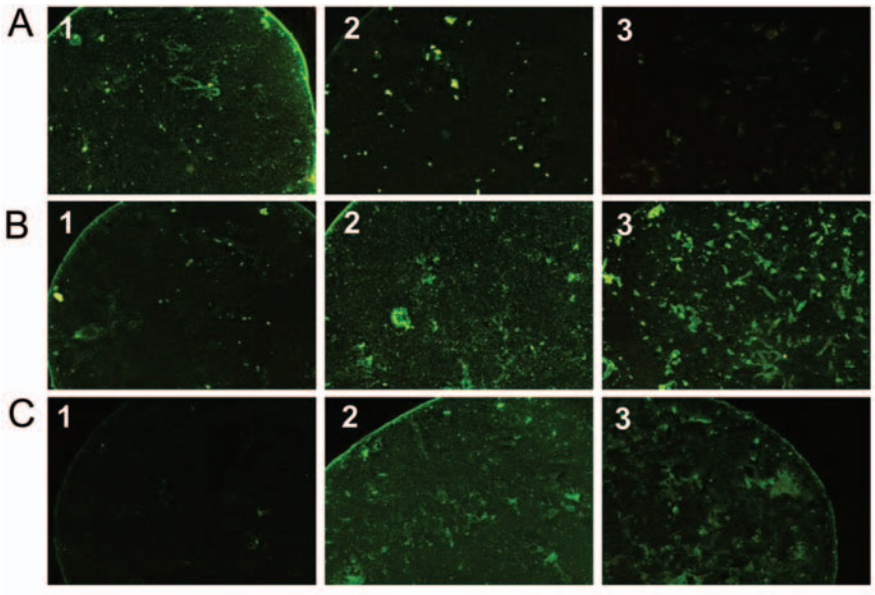

Figure. Pictures of immunofluorescence assay performed on serum specimens with proven *Rickettsia conorii* (A), *R. felis* (B), or *R. typhi* (C) infection showing cross-reactive antibodies. Antigens tested were *R. conorii* (column 1), *R. felis* (column 2), and *R. typhi* (column 3). The serum with *R. conorii* infection reacts with *R. conorii* and *R. felis* antigens but not with *R. typhi* (A). Conversely, the serum with *R. typhi* infection reacts with *R. typhi* and *R. felis* but not with *R. conorii* (C). Finally, the serum with *R. felis* infection reacts with *R. felis*, *R. conorii*, and *R.*

and 1 serum sample was positive for *R. typhi* only. WB with cross-adsorption analysis was performed for 21 serum samples, yielding species-level identification for 19 cases as follows: 3 *R. conorii*, 2 *R. aeschlimannii*, 6 *R. typhi*, and 8 *R. felis*.

Clinical data were available for only 1 patient with *R. aeschlimannii* infection. This patient had fever with meningitis without inoculation eschar or cutaneous rash. Serologic and clinical characteristics were available for 8 patients with *R. felis* infection (Table); most of the patients lived in urban areas, and 1 came from Libya. All 8 patients had fever and a maculopapular rash, and none reported a history of flea or tick bite or had an eschar. Two patients had peripheral adenopathy on admission: cervical and inguinal in the first patient and axillary and inguinal in the second patient.

Conclusions

In this study, IFA and WB identified *R. felis* infection in patients from Tunisia. Serologic cross-reactions are common among *Rickettsia* species in both the SFG and TG. A difference in specific IgG or IgM antibody titers has been useful for distinguishing murine typhus from epidemic typhus (*8*). More sophisticated serologic methods are needed to identify the causative agent at the species level (*9*). WB performed on 7 of 18 serum samples with cross-reactions between SFG, *R. felis*, and *R. typhi* confirmed the diagnosis of *R. felis* in 5 patients and TG rickettsia in 1 patient; the diagnosis remained undetermined in 1 patient. Recently, the whole genome of *R. felis* has been sequenced and demonstrated genetic similarity with *R. typhi* but showed some genes missing from the *R. conorii* genome (*2*). Thus, *R. felis* may be the major cause of cross-reactions between *R. typhi* and *R. conorii* or other tick-borne spotted fever agents. Cross-reactions between the 2 groups of *Rickettsia* have been puzzling because this activity is not reported in experimentally infected guinea pigs and mice (*10*). In fact, we speculate that many of the reactions with both *R. typhi* and *R. conorii* are caused by *R. felis* infection. This hypothesis is supported by our findings of cross-reactivity in serum specimens from 5 of 7 patients with confirmed *R. felis* infection. Indeed, when antigens are not available, this cross-reactivity should be a good screening method for *R. felis* infection. Alternatively, all serum specimens exhibiting cross-reactivity between *R. typhi* and *R. felis* only were considered to be TG rickettsia infection after WB.

To the best of our knowledge, this is the first report of patients with *R. felis* and *R. aeschlimannii* infections in Tunisia. In Morocco, similar results have been reported (*11*). Several cases of SFG rickettsioses have been reported from North Africa, including 1 patient with *R. aeschlimannii* infection from Morocco (*12*) and 1 patient with *R. sibirica mongolitimonae* infection from Algeria (*13*). These results are not surprising since vectors of *R. felis* and *R. aeschlimannii* are present in North Africa (*14*). Indeed, *R. aeschlimannii* has been isolated from *Hyalomma marginatus* ticks collected from camels in Morocco (*15*), and *R. felis*–infected fleas in Algeria have been recently reported (*14*). Since *R. felis* has a worldwide distribution and infestation with these fleas is very common, *R. felis* and fleaborne spotted fever may occur worldwide.

Only a few human cases of *R. felis* infection diagnosed by serologic tests or PCR have been reported: 1 case from the United States (Texas) (*1*), 3 from Mexico (*3*), 2 from France, 2 from Brazil (*4*), and 2 from Germany (*5*). In the

Table. Epidemiologic, clinical, and serologic data for patients with fleaborne spotted fever*

No.	Serologic titers (IgG/IgM)*			Group	Locality	Animal contact	Fever	Eschar	Cutaneous rash	Other signs
	Rickettsia conorii	*R. felis*	*R. typhi*							
2,120	1:128/1:64	1:128/1:16	0/0	2	Sfax	–	40°	No	MP	No
2,274	1:256/1:256	1:512/1:512	0/0	2	–	–	–	–	–	–
2,275	1:256/1:256	1:512/1:512	0/0	2	Moknine	Sheep/ cows	40°	No	No	No
2,147	1:128/1:128	1:1,024/ 1:1,024	1:128/1:1,024	3	Sousse	Dogs/ birds	40°	No	MP	Interstitial pneumopathy
2,245	1:256/1:64	1:1,024/1:512	1:128/1:512	3	Sfax	Cats/ sheep	40°	No	MP	Adenopathy
2,608	1:512/1:64	1:1,024/1:256	1:1,024/0	3	Sfax	No	Yes	No	MP	Adenopathy
2,547	1:32/1:32	1:512/1:256	1:256/1:128	3	Libya	No	Yes	No	MP	
2,421	1:128/1:32	1:512/1:128	1:256/0	3	Sfax	No	Yes	No	MP	

*Ig, immunoglobulin; MP, maculopapular.

Texas case (*1*), the patient had clinical features similar to those associated with murine typhus. However, patients with *R. felis* and central nervous system and pulmonary involvement have been reported from Mexico (*3*). In our study, 1 of the patients with *R. felis* infection had pulmonary involvement and 2 had adenopathy. Although none had an eschar or a history of flea bite, 3 patients had contact with animals.

Our findings indicate the need for further studies to determine the distribution of *R. felis* and the prevalence of this agent and associated infection. These results suggest that fleaborne spotted fever, as well as other SFG rickettsioses, are common in Tunisia.

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Dr Znazen is a medical doctor in the Laboratoire de Microbiologie, Sfax, Tunisia, and is currently studying at the Unité des Rickettsies, Marseilles, France. She is involved in laboratory diagnosis of infectious diseases including human rickettsioses.

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Address for correspondence: Didier Raoult, Unité des Rickettsies, CNRS UMR 6020 Université de la Méditerranée, Faculté de Médecine, Boulevard Jean Moulin, 13385 Marseille CEDEX 05, France; fax: 00-33-4-91-38-77-72; email: Didier.Raoult@medecine.univ-mrs.fr

Genetic Diversity of Sapovirus in Children, Australia

Grant S. Hansman,* Naokazu Takeda,* Kazuhiko Katayama,* Elise T.V. Tu,†‡ Christopher J. McIver,†‡ William D. Rawlinson,†‡ and Peter A. White†

Sapovirus was detected in 7 of 95 stool specimens from children with gastroenteritis of unknown etiology in Sydney, Australia, from August 2001 to August 2002 and from February 2004 to August 2004, by using reverse transcription–polymerase chain reaction. Sequence analysis of the N-terminal capsid region showed all human sapovirus genogroups.

Sapovirus (SaV), a member of the genus *Sapovirus* in the family *Caliciviridae*, is an etiologic agent of human gastroenteritis. SaV-associated infections can cause both mild and acute gastroenteritis. Symptoms include watery stool, mild and or acute diarrhea, stomach cramps, nausea, and vomiting (*1*). In a recent study, the independent risk factor for SaV gastroenteritis in children was contact with an index case-patient, usually in daycare centers (*2*). The most widely used method of SaV detection is reverse transcription–polymerase chain reaction (RT-PCR), which has high sensitivity and can be used for further genetic analysis (*3*,*4*). SaV strains can be divided into 5 genogroups (GI, GII, GIII, GIV, and GV), of which GI, GII, GIV, and GV strains infect humans, while GIII strains infect pigs (*5*,*6*). The 4 human genogroups can be further divided into genotypes (*7*). The purpose of this study was to describe sapovirus-associated infections in Australia.

The Study

We screened stool specimens for SaV by using RT-PCR and described the genetic diversity of virus-positive specimens. A total of 95 stool specimens were collected from children <18 years of age treated for gastrointestinal illness at the Sydney Children's Hospital. Stool specimens were obtained from patients with gastroenteritis of unknown origin despite extensive investigation. These specimens were negative for common foodborne bacterial pathogens (*Salmonella*, *Shigella*, and *Campylobacter*) and enteric viruses (rotavirus, adenovirus, astrovirus, and norovirus) (IDEIA enzyme-linked immunosorbent assay, Dako Cytomation, Ely, UK). The specimens included 67 of 110 specimens obtained from children hospitalized between August 2001 and August 2002 and 28 of 60 specimens from outpatients between February 2004 and August 2004. Specimens were not tested for the presence of SaV if an etiologic agent was already identified (n = 75).

RNA was extracted and purified as described elsewhere (3). Ten microliters of RNA was reverse transcribed by using SuperScript III RNaseH (–) reverse transcriptase according to the manufacture's instructions (Invitrogen, Carlsbad, CA, USA). PCR was conducted by using a nested approach with primers directed against the N-terminal capsid region (*4*). The PCR products were analyzed by 2% agarose gel electrophoresis and visualized by staining with ethidium bromide. PCR-generated amplicons were excised from the gel and purified by using the QIAquick gel extraction kit (Qiagen, Hilden, Germany). Nucleotide sequences were determined by using the terminator cycle sequence kit (version 3.1) and the ABI 3100 Avant sequencer (Perkin-Elmer ABI, Boston, MA, USA). Nucleotide sequences were aligned by using ClustalX, and distances were calculated by using the 2-parameter method of Kimura (*8*). Phylogenetic trees with bootstrap analysis from 1,000 replicas were generated by using the neighbor-joining method as described previously (*8*).

SaV was detected in 7 of 95 stool specimens from children 9 months to 7 years of age with previously unknown causes of acute gastroenteritis. This represented a minimum prevalence of 4.1% (7 of 170 specimens). Sequence analysis showed the presence of all known human SaV genogroups (Figure). Two sequences (strains Sydney31 and Sydney40), which shared ≈99% nucleotide (nt) identity and belonged to genogroup GI, closely matched (>99% nt identity) the Manchester sequence. Three sequences (strains Sydney53, Sydney77, and Sydney4106) that belonged to genogroup GII had 69%–77% nt identity. Sydney4106 had ≈98% nt identity with the Mc10 sequence, Sydney53 had ≈90% nt identity with the C12 sequence, and Sydney77 had ≈99% nt identity with the Bristol sequence.

We recently reported SaV strains Mc10 and C12 as recombinant strains (*7*). Phylogenetic analysis of the nonstructural region (i.e., genome start to capsid start) grouped Mc10 and C12 in 1 GII cluster (*7*), and the structural region (i.e., capsid start to genome end) grouped Mc10 and C12 into distinct GII genotypes (*7*). Evidence suggested that the recombination site occurred at the polymerase and capsid junction in open reading frame 1, as we recently described with recombinant norovirus strains (*9*). Further sequence analysis of the nonstructural region (i.e., 800 nt of the polymerase gene) showed that Sydney4106 had ≈99% nt identity with Mc10, and Sydney53 had ≈92% nt

*National Institute of Infectious Diseases, Tokyo, Japan; †University of New South Wales, Sydney, New South Wales, Australia; and ‡Prince of Wales Hospital, Sydney, New South Wales, Australia

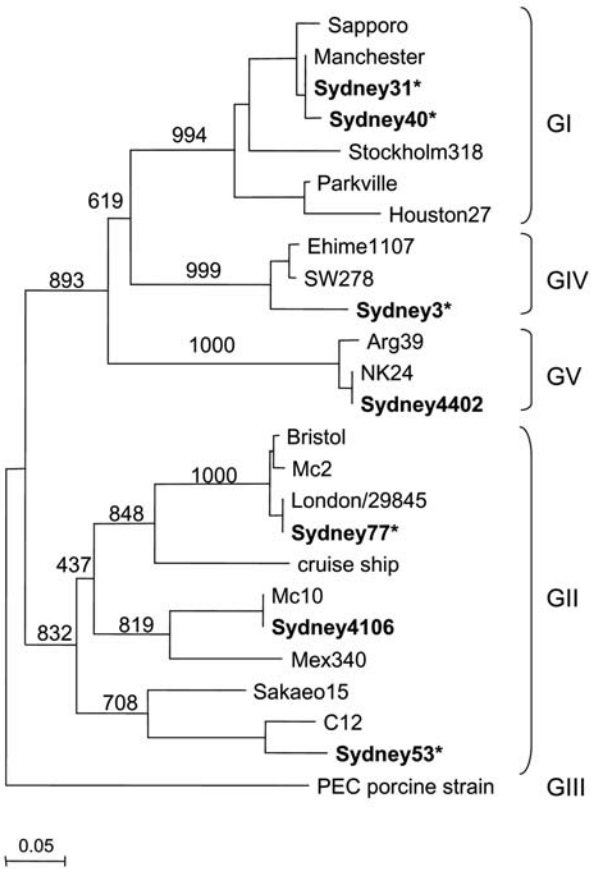

Figure. Phylogenic tree of Sapovirus (SaV) sequences isolated in this study (represented in **boldface**). SaV nucleotide sequences were constructed with the partial N-terminal capsid region, using SaV PEC strain (a porcine SaV) as an outgroup. The numbers on the branches indicate the bootstrap values for the clusters. Bootstrap values >950 were considered statistically significant for the grouping (8). Asterisks indicate specimens collected from outpatients. The distance scale in nucleotide substitutions per position is shown. Sydney isolates have been deposited in GenBank (accession nos. DQ104357–DQ104363). GenBank accession numbers for the reference strains are as follows: Arg39, AY289803; Bristol/98, AJ249939; C12, AY603425; cruise ship/2000, AY289804; Ehime1107, DQ058829; Houston/27/90, U95644; London/29845/90, U95645; Manchester, X86560; Mc2, AY237419; Mc10, AY237420; Mex340/1990, AF435812; NK24, AY646856; Parkville, U73124; PEC, AF182760; Sapporo/82, U65427; Stockholm/318/97, AF194182; Sakaeo15, AY646855; and SW278, DQ125333.

identity with C12. These findings suggest that Sydney4106 and Sydney53 were also recombinant strains and indicate the widespread distribution and genetic stability of recombinant SaV strains. One sequence (strain Sydney3) belonged to genogroup GIV and had ≈99% nt identity with the SW278 sequence, which recently caused an outbreak of gastroenteritis in adults in Sweden in March 2004 (*1*). Another sequence (strain Sydney4402) belonged to genogroup GV and had 100% nt identity with the NK24 sequence, which was isolated from an infant with gastroenteritis in Thailand in December 2002 (*10*). White blood cells were detected in the stool specimens of 3 children infected with SaV genogroups GII, GIV, and GV (strains Sydney4106, Sydney3, and Sydney4402, respectively). In our previous study (*10*), an infant infected with NK24 (SaV genogroup GV) had a fever for 11 days and vomiting for 3 days, which was notably longer than the duration of symptoms in other infants infected with SaV GI and GII strains (unpub. data). These results suggest that some SaV genogroups could be more virulent than others, although additional studies are needed.

Conclusions

Little is known about SaV infections in Australia (*11–14*). Data from these reports indicate that SaV is an uncommon cause of acute gastroenteritis in Australia. When the proportion of SaV present in the total calicivirus isolations was used, SaV was estimated to be the etiologic agent of gastroenteritis in 0.56% (*11*), 0.32% (*12*), and 0.46% (*14*) of cases. Our results have shown that SaV is an important cause of acute gastroenteritis in children in Sydney, with a minimum prevalence of 4.1%, which is higher than previously reported. This is the first report of SaV GIV genogroup-associated infection in Australia and widespread distribution of SaV. However, a more comprehensive study is needed to determine whether predominant SaV strains are circulating, as observed with noroviruses (*7*,*11*,*15*).

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Dr Hansman is a researcher at the National Institute of Infectious Diseases, Japan. His research interests include viruses that cause gastroenteritis in humans, molecular epidemiology of sapoviruses and noroviruses, expression of caliciviruses, and serologic cross-reactivity between viral genotypes.

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Address for correspondence: Grant S. Hansman, Department of Virology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashi-murayama, Tokyo 208-0011, Japan; fax: 81-42-561-4279; email: ghansman@nih.go.jp

Influenza, Winter Olympiad, 2002

Adi V. Gundlapalli,* Michael A. Rubin,* Matthew H. Samore,*† Bert Lopansri,* Timothy Lahey,* Heather L. McGuire,* Kevin L. Winthrop,‡ James J. Dunn,§ Stuart E. Willick,* Randal L. Vosters,¶ Joseph F. Waeckerle,# Karen C. Carroll,*§ Jack M. Gwaltney Jr, Frederick G. Hayden,** Mark R. Elstad,*† and Merle A. Sande***

Prospective surveillance for influenza was performed during the 2002 Salt Lake City Winter Olympics. Oseltamivir was administered to patients with influenzalike illness and confirmed influenza, while their close contacts were given oseltamivir prophylactically. Influenza A/B was diagnosed in 36 of 188 patients, including 13 athletes. Prompt management limited the spread of this outbreak.

The Olympics are the quintessential organized sport where elite international athletes live in close quarters and compete in an intense environment. Upper respiratory illnesses occur frequently (*1*), and influenzalike illnesses (ILI) have been reported in previous Olympics (*2*–*6*). Prospective surveillance was conducted for influenza, with an emphasis on diagnosis, treatment, and prevention, during the 2002 Winter Olympics/Paralympic Games.

The Study

This study was performed at the Olympic Village Polyclinic during the 2002 Winter Olympiad in Salt Lake City, Utah, USA, during February and March 2002. Athletes and nonathletes with upper/lower respiratory symptoms (with or without febrile/systemic illness) were screened for influenza by various modalities. Viral test results from the Polyclinic and public health reports of influenza in the local community were reviewed daily. Patients with ILI or confirmed influenza were offered treatment with oseltamivir; close contacts were offered prophylaxis (detailed methods available from corresponding author by email).

A total of 2,635 medical visits were recorded during the Games; patients with any respiratory symptom represented 12%. Of these, 188 satisfied the symptom criteria for the study (available from corresponding author) and were screened for influenza (Table 1). Influenza A was detected in 28 (15%) and influenza B in 8 (4%) patients (Table 2). Athletes comprised 36% of all influenza patients. Of the influenza A isolates, 8 were further analyzed and found to be consistent with the A/Sydney/ 97(H3N2) strain (represented in the 2001–2002 vaccine).

Patients with confirmed influenza (Table 2) were more likely to be male, have a temperature ≥37.8°C, and have a history of cough or chills. No significant differences were found in symptom duration or influenza vaccination status among those with and without influenza. Athletes were more likely to have a diagnosis of influenza A than other pooled groups of nonathletes (odds ratio [OR] 3, 95% confidence interval [CI] 1.1–7.5, p = 0.03).

Twenty-five of 188 patients who were screened by direct fluorescent-antibody assay (DFA) for influenza were positive. When the results were compared to viral culture alone, sensitivity was 70%, specificity was 99%, positive likelihood ratio was 54, and negative likelihood ratio was 0.3. Ten (6%) of the 160 who received a rapid influenza test had positive results. The sensitivity of the rapid test for diagnosing influenza (when compared to a confirmed diagnosis by viral culture, polymerase chain reaction, or DFA) was 17%, while the specificity was 97%. The positive likelihood ratio and negative likelihood ratio were 5.2 and 0.9.

The conventional syndromic definition of ILI (fever and either cough or sore throat) (*7*) had a low positive likelihood ratio of 2.7, negative likelihood ratio of 0.5, sensitivity of 67%, and specificity of 78% in predicting influenza. Overall, 23% of nonathletes and 18% of athletes screened reported influenza vaccination. Of those with confirmed influenza, vaccinees were likely to have lower fevers, although the results were not significant.

Physicians prescribed oseltamivir for 60 (32%) of 188 patients screened for influenza. Of the medicated patients, 40 (67%) were treated for ILI within 48 hours of symptom onset; influenza was confirmed in 21. Oseltamivir prophylaxis (for 5 days) was prescribed in 20 (33%) patients who had a history of contact with influenza patients; 1 case of influenza was confirmed in this group. All patients who received oseltamivir tolerated the medication well.

Three distinct clusters of ILI were identified during the Games. Cluster I consisted of 13 law enforcement personnel who worked and lived in close proximity. In early February, 3 members came to the clinic 4 days apart with ILI, and influenza A was diagnosed (2 cases by DFA, 1 by viral culture). Oseltamivir prophylaxis was promptly initiated in the remaining 10 asymptomatic members; the oseltamivir was well tolerated. No other cases of ILI were reported. The group was able to discharge its duties in the village.

Cluster II consisted of 12 members of a national team who had trained together at a common location 3 days

*University of Utah School of Medicine, Salt Lake City, Utah, USA; †Veterans Affairs Medical Center, Salt Lake City, Utah, USA; ‡Centers for Disease Control and Prevention, Atlanta, Georgia, USA; §ARUP Laboratories, Inc., Salt Lake City, Utah, USA; ¶Lakeshore Medical Clinic, Milwaukee, Wisconsin, USA; #Kansas City School of Medicine, Kansas City, Missouri, USA; and **University of Virginia, Charlottesville, Virginia, USA

Table 1. Patients screened for influenza, 2002 Winter Olympic and Paralympic Games

Characteristic	Olympics, n (%)	Paralympics, n (%)
No. patients screened	156	32
No. countries represented	45	9
Age, y, mean (range)	34 (18–67)	37 (20–65)
Sex, male	98 (63)	15 (47)
History of influenza vaccination before arrival	37 (24)	7 (22)
Accreditation		
Organizing committee volunteers and staff	62 (40)	21 (65)
Athletes	41 (26)	5 (16)
Law enforcement personnel	29 (19)	1 (3)
Olympic family	24 (15)	5 (16)
Tests performed*		
Direct fluorescent antibody and viral culture	156 (100)	32 (100)
Rapid streptococcal antigen test	98 (63)	12 (38)
Rapid influenza test	141 (90)	19 (59)
Multiplex reverse transcription–polymerase chain reaction for respiratory viruses	33 (21)	4 (13)

*All patient specimens were screened for influenza by direct fluorescent antibody (DFA) and viral culture. Selected samples that were negative by DFA and viral culture were screened by reverse transcription–polymerase chain reaction for influenza. Rapid tests for influenza and streptococcal antigen were conducted on selected patients based on their symptoms and the clinician's discretion. Detailed methods are available from the corresponding author by email.

before their arrival at the Olympic Village. Two days after they arrived, the index patient (unvaccinated for influenza) came to the clinic with ILI of 24 hours' duration and was given oseltamivir. Upon confirmation of influenza A by DFA, unvaccinated asymptomatic close contacts of the patients were offered oseltamivir prophylaxis; 8 of 11 accepted. In the next 4 days, 3 vaccinated teammates who had not received prophylaxis came to the clinic with ILI of 24 hours' duration. Treatment was initiated because of their close contact with the index patient. One patient was subsequently found to have influenza A by DFA. No further cases of ILI were reported. The team competed successfully in the sport and won several medals.

Cluster III consisted of 8 participants of 1 sport (which had 80 participants with common training venues) sought treatment at the Polyclinic within 9 days with respiratory symptoms (5 had ILI, 3 were afebrile). The 5 with ILI were treated with oseltamivir. Of the 3 afebrile participants, 2 were provided prophylaxis based on their contact history and symptoms. The third patient was not offered prophylaxis due to insufficient contact history. Influenza A was confirmed in 5 patients. No reports of ILI or confirmed influenza occurred among participants from this group after treatment/prophylaxis was initiated.

Conclusions

This is the first systematic influenza study at any large international sports gathering and demonstrates the

Table 2. Patients treated, 2002 Winter Olympic and Paralympic Games

Characteristic	Influenza A or B	Noninfluenza	Odds ratio (95% CI*), p adjusted
No. patients treated	36	152	
Accreditation (%)			
Organizing committee volunteers and staff	14 (39)	69 (45)	Reference
Athletes	13 (36)	33 (22)	2 (0.8–4.6), p = 0.1†
Law enforcement personnel	4 (11)	26 (17)	1.3 (0.2–2.5), p = 0.7
Olympic family	5 (14)	24 (16)	1 (0.3–3.2), p = 0.9
Age, y, mean (standard deviation [SD])	32 (10)	35 (13)	0.7 (0.4–1.0), p = 0.08‡
Sex, male %	78	56	5.5 (1.6–18.3), p = 0.006
Symptom duration, mean/median days (SD)	2.9/2 (3.5)	3.7/2 (5)	2.0 (0.7–5.8), p = 0.2§
History of influenza vaccination (%)	7 (19)	37 (24)	1.7 (0.5–1.6), p = 0.4
Temperature ≥37.8°C (%)	14 (39)	7 (5)	13 (4.7–36), p<0.001
Symptoms (%)			
History of fever	22 (61)	40 (26)	1.2 (0.4–3.7), p = 0.8
Cough	33 (92)	90 (59)	25.7 (2.2–155), p<0.001
Chills	20 (56)	33 (22)	3.9 (1.2–12.8), p = 0.02
Myalgia	23 (64)	57 (38)	2.1 (0.7–6.4), p = 0.2
Sore throat	22 (61)	110 (72)	0.4 (0.1–1.4), p = 0.2

*CI, confidence interval.
†Athletes are a significant group when influenza A cases alone are considered, odds ratio 3, 95% CI (1.1–7.5) p = 0.03.
‡Age as grouped by decade.
§Symptom duration was grouped as ≤48 h or >48 h.

feasibility of managing influenza at such events. The intervention strategy integrated a policy of empiric treatment based on clinical data and viral testing with a public health surveillance approach, including daily review of all viral test results from the Polyclinic and reports of influenza in the community. Potential clusters of influenza were promptly identified, index patients were treated with oseltamivir, and contacts were given oseltamivir prophylaxis.

We examined several methods of detecting influenza from respiratory samples and found DFA testing to be the most useful surveillance tool in this setting. The sensitivity of rapid testing was low. This observation is consistent with the variability typically associated with rapid testing regarding patient age, duration of symptoms, type of kit, and timing of specimen acquisition (*7–9*).

A low rate of influenza immunization was noted among participants. The World Health Organization and others have suggested that vaccination is beneficial for athletes (*2,4,10–12*). Although this study was not designed to address the effectiveness of influenza vaccination, we support issuing a public health alert that encourages administering influenza vaccine to all athletes and staff before a large international event is staged.

Team physicians may not have reported all episodes of ILI to the Polyclinic, though this scenario is unlikely, given their frequent direct communication. Alternative strategies for influenza control, such as mass vaccination (*13*), were not examined in this study. Followup was not attempted since patients often dispersed to various international destinations after their events.

In summary, the surveillance and intervention strategy used in this study may serve as a model for mobilizing teams to provide health care to a large assembly of participants. Initiating empiric treatment for influenza based on clinical and epidemiologic data, combined with testing by DFA (with subsequent confirmation by viral culture), may be a prudent approach to influenza control in large gatherings. Close contacts of persons with positive DFA tests would then be candidates for prophylaxis. Similar approaches may enhance preparedness for public health threats and emerging respiratory pathogens such as avian influenza and agents of bioterrorism.

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Dr Gundlapalli is an assistant professor of medicine in the Division of Infectious Diseases at the University of Utah School of Medicine and medical director of Wasatch Homeless Health Care, Inc., in Salt Lake City, Utah. His research interests include public health surveillance, biodefense, emerging infections, and healthcare for the homeless.

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Address for correspondence: Adi V. Gundlapalli, Division of Infectious Diseases, Room 4B319 SOM, University of Utah School of Medicine, 30 North 1900 East, Salt Lake City UT 84132 , USA; fax: 801-585-3377; email: adi.gundlapalli@hsc.utah.edu

Novel Human Metapneumovirus Sublineage

Barbara Huck,* Gesa Scharf,*
Dieter Neumann-Haefelin,* Wolfram Puppe,†
Josef Weigl,† and Valeria Falcone*

In a pediatric surveillance network, 287 (5.1%) of 5,580 specimens from patients with acute respiratory infections tested positive for human metapneumovirus (HMPV). Phylogenetic analysis of N- and F-gene sequences of identified HMPV showed that 30% belonged to a novel phylogenetic cluster.

Human metapneumovirus (HMPV), a newly discovered member of the family *Paramyxoviridae*, pneumovirus subfamily, was first isolated in the Netherlands in 2001 (*1*). Since then, a variety of reports have confirmed the worldwide prevalence of HMPV and identified this virus as an important respiratory pathogen in young children (*2*–*5*). Sequence analysis of several isolates has identified 2 major genetic lineages (subtypes A and B) that can be divided into subgroups A1, A2, B1, and B2 (*6*,*7*). Since a growing number of sequence data are available, we initiated a systematic analysis of HMPV genotypes within subtypes A and B to better characterize HMPV variability.

The Study

From October 2002 to June 2004, nasopharyngeal aspirates (NPAs) from 5,580 pediatric patients ≤16 years of age (median 22 months) were collected by a pediatric infectious diseases network on acute respiratory infections (PID-ARI.net). This surveillance system comprises 3 study areas in northern (Kiel), midwestern (Mainz), and southern (Freiburg) Germany. Two percent of the total German pediatric population are under surveillance. Those identified by this network included hospitalized children (87.6%) and outpatients (12.4%) with symptoms of acute upper or lower respiratory tract infections. The most common signs and symptoms were cough, rhinorrhea, pharyngitis, otitis media, exanthema, fever, rales, and retractions.

Respiratory pathogens were detected by multiplex reverse transcription–polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay, based on methods described previously (*8*), including primers specific for HMPV, respiratory syncytial virus, parainfluenza viruses 1–3, rhinoviruses, influenza A and B viruses, adenoviruses, and enteroviruses, as well as noncolonizing bacterial pathogens of the respiratory tract. The primers used for HMPV detection correspond to those published previously (*9*). The L-gene primers were used from October 2002 to June 2003; the N-gene primers, adapted versions of the published (NL-N) primers, were used from July 2003 to June 2004. HMPV was detected in 287 NPAs: 51 (1.9%) of 2,599 specimens in the first season (October 2002–June 2003), and 236 (7.9%) of 2,981 specimens in the second season (July 2003–June 2004). RNA was extracted by using the QIAamp Viral RNA Mini-Kit (Qiagen GmbH, Hilden, Germany). Two independent regions of the nucleocapsid (N, nucleotide [nt] 454–878) and fusion protein gene (F, nt 3,624–4,130) were selected for phylogenetic analysis and amplified with the primer pairs: N-f (5′-CCYTCAGCACCAGACACACC-3′), N-r (5′-AGATTCAGGRCCCATTTCTC-3′) and F-f (5′-GTYAGCTTCAGTCAATTCAACAGAAG-3′), F-r (5′-CCTGTGCTGACTTTGCATGGG-3′) by using the Qiagen OneStep RT-PCR Kit (Qiagen GmbH). We used 5 μL RNA in a volume of 50 μL, a primer concentration of 0.6 mmol/L, and the following reaction conditions: 30 min at 50°C, 15 min at 95°C, 35 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, and a final incubation of 10 min at 72°C. Nucleotide sequences from amplified F and N gene products purified with the QIAquick PCR Purification Kit (Qiagen GmbH) were determined by using the BigDye V 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) on an automated ABI 3730 XL capillary sequencer (GATC, Konstanz, Germany). Sequences were aligned with prototype HMPV strains from Canada and the Netherlands (GenBank accession numbers AY297748, AY145295, AY145277, AY297749, AY355324, AY145301, AY145276, AY145299, AY145274, AY145294, AY145286, AY355335, AY525843, AF371337, AY530095) and sequences of the avian metapneumovirus C (AY590688), by using the ClustalW algorithm of the Megalign software (Lasergene, DNA Star, Madison, WI, USA). Neighbor-joining trees were generated with neighbor-joining and the Kimura 2 parameter substitution model by using MEGA software (*10*); 1,000 bootstraps were performed on the neighbor-joining trees. Additional phylogenetic testing of the datasets was performed by maximum likelihood (ML) analysis with the DNAml software of PHYLIP (PHYLIP [Phylogeny Inference Package] Version 3.6; distributed by J. Felsenstein, Department of Genome Sciences, University of Washington, Seattle, WA, USA). One hundred bootstrap replicates were calculated on the resulting phylogenetic trees by using the Seqboot and Consense programs of the PHYLIP package. The datasets for the 2 seasons were analyzed separately. Partial N-gene and F-gene sequences of 424 and 506 nt, respectively, were obtained for 230 NPA

*University Hospital Freiburg, Freiburg, Germany; and †University Hospital Schleswig-Holstein, Kiel, Germany

samples. In 57 samples that initially tested positive for HMPV, the viral genotype could subsequently not be determined because of insufficient NPA, unsuccessful recovery of viral RNA after shipping and extended storage, or differences in the extraction methods and RT-PCR.

Phylogenetic analysis of the N-gene fragment by the neighbor-joining method performed on 191 samples from the season 2003–2004 (Figure 1B) confirmed the existence of 2 main genetic lineages, A and B, and the 2 formerly reported subgroups, B1 and B2, in lineage B. However, lineage A appeared to consist of 3 subclusters (Figure 1). In fact, we found 2 clusters, tentatively named A2a and A2b, within the formerly reported subgroup A2. This partition of subgroup A2 is supported by high bootstrap values (94% and 98%) comparable to those found for the widely accepted partition of A into A1 and A2. When these sequences were compared in an ML analysis (data not shown), they showed the same tree topology, again supported by high bootstrap values (86%). Moreover, neighbor-joining analysis of the F-gene fragment confirmed the observed partition within the A2 subgroup (Figure 1B) sustained by similar bootstrap values. Therefore, the proposed classification is independent of the calculation model and valid for both gene fragments tested. Analysis of the 39 samples from the 2002–2003 season showed the same tree topology by both neighbor-joining and ML analysis (data not shown). Thus, the cluster A2b was consistently prevalent within 2 consecutive seasons.

The analysis of sequence similarity additionally supported the observed tree topology. For the F-gene fragment, nucleotide identity between groups A and B was 83.6%–87.4%, whereas it was 92.1%–94.3% and 94.0%–95.7% between subgroups A1–A2 and B1–B2, respectively. The sequences within the 3 subgroups A1, B1, and B2 shared a nucleotide identity of 97.1%–99.5%, 97.1%–99.8%, and 98.3%–99.5%, respectively. Reflecting the tree topology, subgroup A2 was the most divergent; sequences shared 92.1%–99.8% nucleotide identity in the F gene. Within clusters A2a and A2b, nucleotide identity of 99.3%–99.8% and 97.1%–99.8% was found, thus confirming the existence of 2 genetically distinct clusters, A2a and A2b. Analysis of the sequences obtained for the N gene yielded comparable results (data not shown).

Phylogenetic analysis of previously published sequences of the HMPV F and N genes of strains from various countries showed that the subtype A sequences cluster

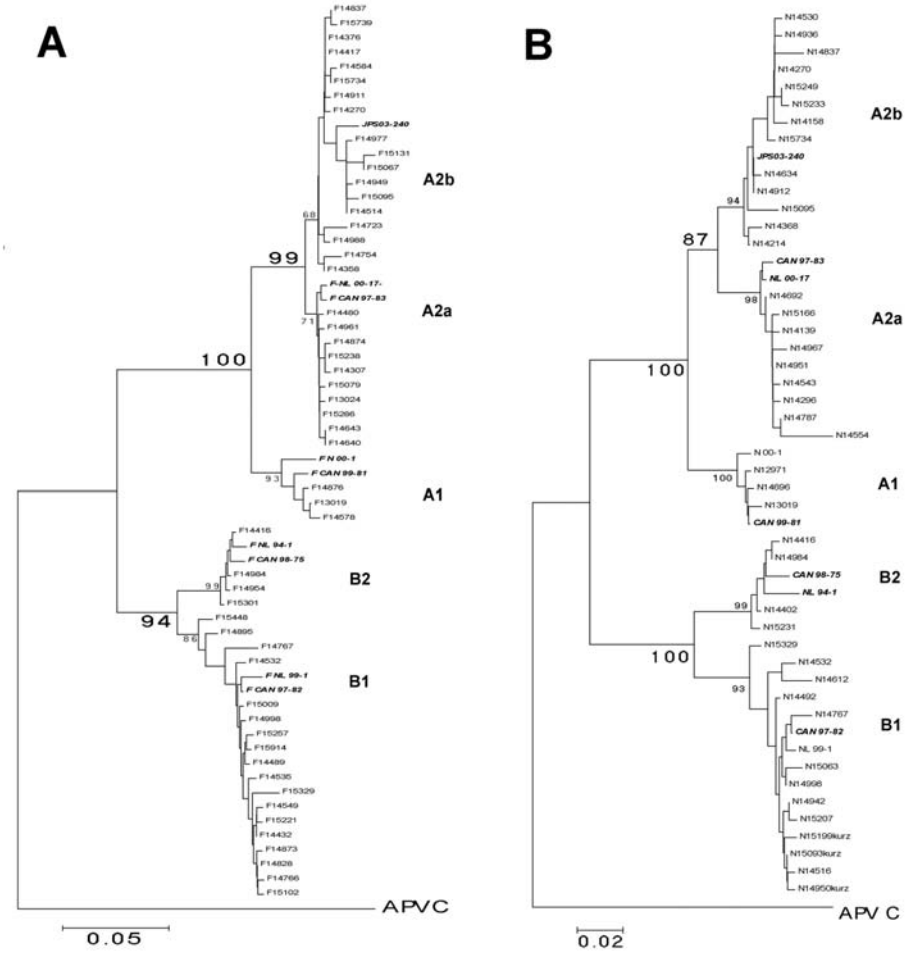

Figure 1. Neighbor-joining phylogenetic trees of human metapneumovirus (HMPV). A) Partial F gene (506-nucleotide [nt] fragment). B) Partial N gene (424-nt fragment) of 191 HMPV strains recovered in Germany during the 2003–2004 season. Bootstrap resampling was applied (n = 1,000) with random sequence addition. Bootstrap values based on the consensus tree are plotted at the main internal branches to show support values. Sequences from the avian metapneumovirus C were included in the analysis and used as outgroup. Isolates from the Netherlands, Japan, and Canada were additionally included in the analysis.

with few exceptions in subgroups A2a and A1. None of the fully sequenced prototype isolates (NL 00-1, CAN 98-75, CAN 97-83, NL 99-1) is part of the new cluster A2b. Only 4 isolates from Japan, e.g., JPS03-240 shown in Figure 1 (*11*), were assigned to A2b. In other studies, A2b isolates may have been missed because very short fragments were sequenced (*1*,*3*,*12*). In contrast, 70 (30%) of 230 specimens analyzed in this study belonged to cluster A2b.

When we compared the prevalence of the different genotypes during the 2 seasons, we found that HMPV genotypes A1 and B2 were marginally present in both seasons and that the major portion consisted of the A2 and B1 genotypes (Figure 2A). Group A strains were more prevalent than group B strains, in particular, during 2002–2003. Differentiation of the hospitalized patients and the outpatients did not show any significant differences in genotype distribution among the 2 groups. However, we cannot rule out the existence of such a difference because of the small percentage of outpatients (12.4%).

Year-round sampling and testing confirmed a seasonal distribution of HMPV-positive cases (*12*). Seasonal peaks were observed from March to July 2003 and from January to March 2004 (Figure 2B). The rates of HMPV and respiratory syncytial virus detection differed significantly between the 2 years and seemed to be inversely correlated (1.9% vs 15.2% in 2002–2003 and 7.9% vs 11.0% in 2003–2004). Differences in distribution and prevalence of HMPV between the 2 seasons must be interpreted with caution for 2 reasons. First, the number of HMPV-positive specimens from the first year is small. Second, the primers used during the first season demonstrated a variable sensitivity for different genetic lineages (*9*). However, our data are consistent with previous observations of high and low incidence of HMPV in alternating years (*13*,*14*). For respiratory syncytial virus,, biannual periodicity with alternating occurrence of minor epidemics (slow onset, low peak, long duration) and major epidemics (rapid onset, high peak, short duration) has been described in temperate climates (*15*). To speculate that HMPV epidemics exhibit a similar pattern of periodicity is tempting, although additional studies performed over longer periods are needed to better define the seasonality of HMPV.

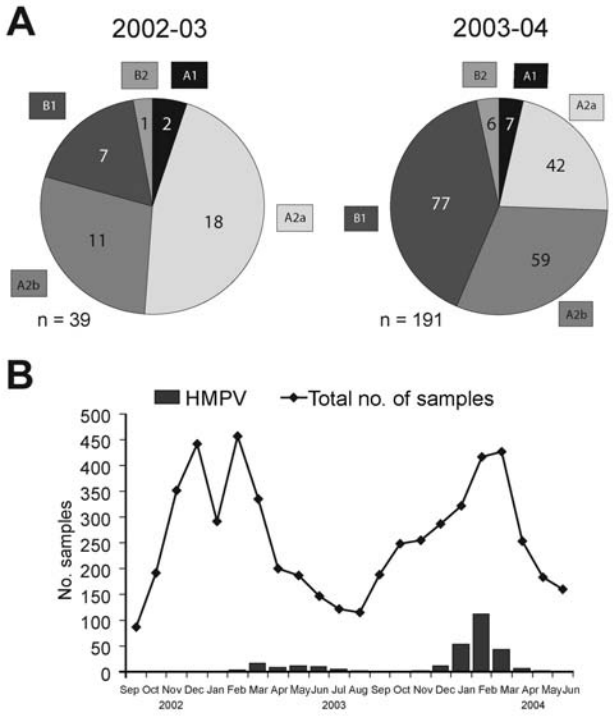

Figure 2. Circulation of human metapneumovirus (HMPV) in Germany in a 2-year period from October 2002 to June 2004. A) Distribution of HMPV genotypes of 39 patients tested during the 2002–2003 season and 191 patients tested during the 2003–2004 season. B) Seasonal distribution of HMPV-infected patients and overall study admissions by month of admission. Further information about other respiratory pathogens cocirculating during the observed season is available from http://www.pid-ari.net

Conclusions

The molecular epidemiology of the HMPV circulating in Germany during 2 consecutive seasons was distinct from the previously proposed classification scheme. By genotyping >200 samples, we confirmed the existence of the 2 main HMPV subtypes A and B and of 4 minor subgroups. However, subgroup A2 was more divergent than reported to date. Phylogenetic analysis of both the F and the N genes showed a further bipartition of subgroup A2. Thus, we identified 2 new genetic clusters, designated A2a and A2b. Comparison of HMPV prevalent in Germany during 2 seasons showed cocirculation of all described genotypes with subtype A predominating over subtype B, thus confirming previous reports (*15*). Within subtype A, subgroup A2 accounted for most cases. Moreover, approximately one third of all genotypes were classified as A2b in both seasons. Given this high prevalence, genotype A2b should be considered in HMPV primer design for diagnostic assays. The finding that sequences from Japanese HMPV isolates belong to genotype A2b (*11*) supports the idea that this novel sublineage is not locally or temporarily restricted but might be prevalent worldwide.

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Dr Huck is a research fellow at the Department of Virology of the University Hospital Freiburg, Germany. Her current

research interest focuses on molecular epidemiology and pathogenesis of respiratory viruses in immunocompromised and pediatric patients.

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Address for correspondence: Barbara Huck, Department of Virology, Institute for Medical Microbiology and Hygiene, Hermann-Herder-Str. 11, 79104 Freiburg, Germany; fax: 0049-761-2036626; email: barbara.huck@uni-heidelberg.de

Novel Parvovirus and Related Variant in Human Plasma

Jacqueline F. Fryer,* Amit Kapoor,†‡ Philip D. Minor,* Eric Delwart,†‡ and Sally A. Baylis*

We report a novel parvovirus (PARV4) and related variants in pooled human plasma used in the manufacture of plasma-derived medical products. Viral DNA was detected by using highly selective polymerase chain reaction assays; 5% of pools tested positive, and amounts of DNA ranged from <500 copies/mL to >10^6 copies/mL plasma.

Using a sequence-independent polymerase chain reaction (PCR) amplification method, we recently identified a new parvovirus in plasma from a patient with exposures and symptoms consistent with acute HIV infection, but who was HIV RNA negative (*1*). Phylogenetic analyses of sequence data suggest that this virus, termed PARV4, is only distantly related to previously known human or animal members of the family *Parvoviridae*, including members of the *Erythrovirus* genus known to infect humans, such as parvovirus B19. Infection with parvovirus B19, although frequently asymptomatic, may result in erythema infectiosum, arthropathy, pregnancy complications (e.g., hydrops fetalis), transient aplastic crisis, and disease in immunocompromised patients (*2*). Parvovirus B19 is most frequently transmitted through the respiratory route or vertically from mother to fetus. However, blood- and plasma-derived medical products, particularly clotting factors, contaminated with parvovirus B19 can also transmit the virus (*3*). Manufacturers of plasma derivatives screen minipools by using nucleic acid amplification techniques (NAT), which has enabled levels of erythrovirus DNA to be substantially reduced in start pools; for certain products, screening is now a regulatory requirement (*4*). This study examined pooled human plasma for fractionation to detect PARV4 DNA sequences.

The Study

Samples of manufacturing plasma pools submitted to the National Institute for Biological Standards and Control for testing for hepatitis C virus RNA were stored at –70°C until analysis, in compliance with European regulatory requirements. Manufacturing pools were sourced from donations collected in Europe and North America and received during the previous 6 months. Total nucleic acid was extracted from plasma pools as described previously (*4*) before analysis for PARV4 DNA.

Using multiple sequence alignments of human erythroviruses and comparison with the sequence for PARV4 (*1*), we designed highly selective primers to the open reading frame 1 (ORF1) of PARV4, homologous to the nonstructural proteins of other parvoviruses. Primers PV4ORF1F (5′-AAGACTACATACCTACCTGTG-3′) and PV4ORF1R (5′-GTGCCTTTCATATTCAGTTCC-3′) amplify a 220-bp region of ORF1. The specificity of these primers was confirmed by PCR using a cloned fragment of the ORF1 region alongside erythrovirus control material (Figure 1A). Each PCR contained 1× PCR buffer II (PE Applied Biosystems, Warrington, UK), 200 μmol/L each deoxynucleoside triphosphate, 2 mmol/L $MgCl_2$, 10 pmol each primer, and 2.5 U AmpliTaq Gold DNA polymerase (PE Applied Biosystems) in a final volume of 50 μL. For thermal cycling, a T3 thermal cycler (Biometra, Göttingen, Germany) was used with the following cycling conditions: 95°C for 9 min, followed by 45 cycles of 96°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Amplicons were analyzed by agarose gel electrophoresis and compared to known size markers. The PARV4 control sequences (nucleotides 1293–1833 of ORF1, GenBank accession no. AY622943) were cloned into the vector pT7 Blue according to the manufacturer's instructions (Novagen, Darmstadt, Germany). The sensitivity of these PCR reactions was 1–10 copies of PARV4 sequences. DNA extracted from 137 pools was screened for PARV4 ORF1 sequences by PCR using 5 μL extracted DNA. Results, summarized in Table 1, show that 7 of 137 plasma pools screened with these primers tested positive for PARV4 DNA sequences and those of a related variant, known as PARV5. Typical results from pools and control plasmid samples are shown in Figure 1B. DNA sequence analysis showed that PARV5, over the region amplified, shares ≈92% nucleotide identity with PARV4 (Figure 2). Further sequence analysis around the primer-binding sites showed that the primers were 100% homologous in both genotypes. This level of relatedness is similar to that seen for the different erythrovirus genotypes (*7*).

The levels of PARV4 in the positive plasma pools were determined by real-time PCR using the screening primers from the ORF1 region of PARV4. Amplification reactions were performed on the LightCycler instrument using the LightCycler FastStart DNA Master^{PLUS} SYBR green I kit (Roche Applied Science, Mannheim, Germany) in accordance with the manufacturers' instructions. A standard

*National Institute for Biological Standards and Control, Hertfordshire, United Kingdom; †Blood Systems Research Institute, San Francisco, California, USA; and ‡University of California, San Francisco, California, USA

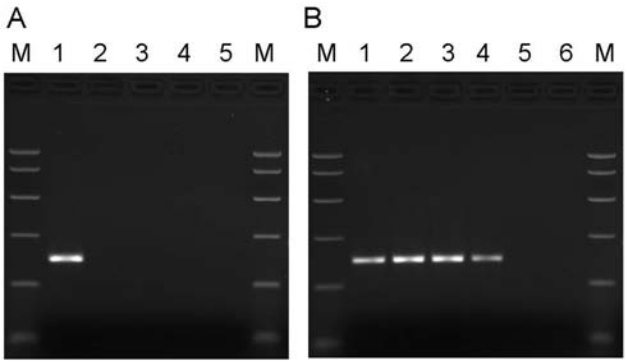

Figure 1. A) Specificity of primers for PARV4. Samples in lanes 1–5 were amplified by using primers directed to open reading frame 1 (ORF1) of PARV4. Template DNA in lane 1 was a plasmid subclone of the PARV4 ORF1 region. In lane 2, the template DNA was derived from parvovirus B19 International Standard (99/800, National Institute for Biological Standards and Control, South Mimms, UK) as representative of genotype 1 erythrovirus sequences; in lane 3, the template DNA was derived from a genotype 2 erythrovirus plasmid clone (A6; obtained from K. Brown, National Heart, Lung and Blood Institute, Bethesda, MD, USA); in lane 4, the template DNA was derived from a genotype 3 erythrovirus plasmid clone (D91.1; obtained from A. Garbarg-Chenon, Hôpital Trousseau, Paris, France). Template DNA in the erythrovirus samples (lanes 2–4) was adjusted to give $\approx 10^{5.5}$ copies of each genotype per reaction. Lane 5, no template control. Polymerase chain reaction (PCR) products were analyzed on a 2.5% agarose gel alongside PCR Markers (M) (Promega, Madison, WI, USA). B) Screening manufacturing plasma samples for PARV4. Samples in lanes 1–6 were amplified by using primers directed to the ORF1 region of PARV4. Template DNA in lanes 1 and 2 consisted of 1×10^2 and 1×10^3 copies of the ORF1 subclone of PARV4. In lane 3, the template DNA was derived from a plasma pool containing 3.9×10^6 PARV4 genome copies/mL plasma; in lane 4, the template DNA was derived from a plasma pool containing <500 PARV4 genome copies/mL plasma; in lane 5, the template DNA was derived from a plasma pool that tested negative for PARV4 sequences. Lane 6, no template control. PCR products were analyzed on a 2.5% agarose gel alongside PCR Markers (M) (Promega).

curve was generated from the cloned plasmid DNA containing the ORF1 fragment of PARV4. Levels of PARV4 DNA were as high as 3.9×10^6 copies/mL plasma, although several pools contained <500 copies/mL plasma (Table 2).

Plasma pools found positive for PARV4 sequences were tested for the levels of erythrovirus DNA as described previously (*4*). Only 2 of the PARV4-positive pools contained any human erythrovirus DNA, and these were at low levels (Table 2). Of the plasma pools found to be positive for PARV4 sequences, blood products from only 2 were available for further analysis. Both products were immunoglobulin preparations, and in neither case could PARV4 sequences be detected.

Table 1. Analysis of plasma pools for PARV4 and PARV5

Manufacturer	No. positive/no. analyzed
A	5/12
B	0/7
C	0/9
D	2/6
E	0/14
F	0/21
G	0/50
H	0/16
I	0/2

Conclusions

This report is the first to describe novel parvovirus sequences in pooled human plasma for fractionation. PARV4 was originally identified in a patient with acute viral infection syndrome coinfected with hepatitis B virus (*1*). As yet, nothing is known about the prevalence of PARV4, its possible role in human disease, or whether PARV4 was transmitted to the original patient from an unidentified animal host.

Although PARV4 shares limited homology with human erythroviruses, the latter are frequent contaminants of plasma, pooled and used for fractionation (*3*). Levels of PARV4 DNA ranged from <500 copies/mL to $>10^6$ copies/mL

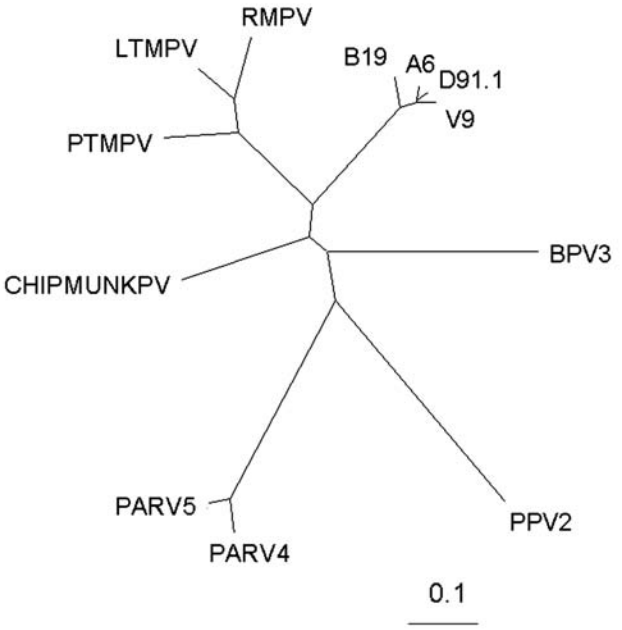

Figure 2. Phylogenetic analysis of a 178-bp sequence of ORF1 of PARV4 and PARV5 (GenBank accession no. DQ112361) with other members of the *Parvoviridae* subfamily. The alignment includes the members of the *Erythrovirus* genus (parvovirus B19 [*5*]) and related viruses such as V9 (*6*), D91.1 (*7*), and A6 (*8*), as well as the closely related viruses infecting the cynomolgus macaque (LTMPV) (*9*) and rhesus (RMPV) and pig-tailed macaques (PTMPV) (*10*). Two other viruses tentatively assigned to the group include a parvovirus isolated from chipmunks (*11*); BPV3, a novel bovine parvovirus (BPV3) (*12*); and porcine parvovirus 2 (PPV2) (*13*). Analysis was performed by using the program ClustalW (*14*).

Table 2. Viral loads in plasma pools that tested positive for PARV4 or PARV5 sequences

Positive pool	Manufacturer	PARV4 viral load (genome copies/mL plasma)	Human erythrovirus viral load (IU/mL plasma)
1	A	5×10^5	Negative
2	D	<500	Negative
3	A	3.9×10^6*	140
4	A	<500*	340
5	A	2.1×10^4*	Negative
6	A	<500*	Negative
7	D	Not determined	Not determined

*Sequences contaminating plasma pool represent PARV5 and not PARV4.

plasma. If a single donation with a high PARV4 count was responsible for the contamination of such a pool, the levels of virus DNA in the original donation would have been in the order of 10^9 or 10^{10} copies/mL plasma, given the volume of the start pool. Because erythroviruses are small, nonenveloped, and relatively resistant to virus inactivation procedures, manufacturers of plasma-derived products have used NAT to exclude high-titer donations from manufacturing start pools. Before such measures were introduced, more than half of production start pools contained erythrovirus DNA, some with titers of 10^9 copies/mL plasma (*4*; S. Baylis, unpub. data). The prevalence of PARV4 and PARV5 and the titers observed in the pools examined in this study are much lower than the usual prevalence and titers observed with erythroviruses. Because of PARV4's insufficient homology with human erythroviruses, current methods of NAT are unlikely to identify donations positive for PARV4.

The availability of highly specific reagents for PARV4 and PARV5 will assist in further studies to elucidate their possible role in human disease. The detection of PARV4 and PARV5 in plasma may have been caused by an epidemic at the time of plasma donation. In a recent study that screened for enteroviruses in human plasma, seasonal changes were observed in the frequency and level of viremia (*15*). Studies to examine the epidemiology of PARV4 and PARV5 infection will help address issues such as these.

In summary, PARV4, a novel parvovirus, and PARV5, a related variant, have been identified in plasma used in the manufacture of blood products. Plasma is obtained from healthy persons, who at the time of donation are asymptomatic, despite being viremic for PARV4 or PARV5. Highly specific and sensitive assays to detect PARV4 will facilitate further analysis of the role of this novel virus in human disease and the implications of virus transmission by contaminated blood and blood products.

Note

After this article was submitted for publication, human bocavirus, a novel parvovirus, was identified in respiratory tract samples (16). PARV4 and PARV5 are distinct from human bocavirus. For example, comparison of PARV4 (AY622943) with human bocavirus strains ST and ST2 (DQ000495 and DQ000496) shows nucleotide identity of 41% and 40%, respectively.

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Dr Fryer is a scientist at the National Institute for Biological Standards and Control. Her work focuses on the quality of blood and blood products with respect to transfusion-transmitted infections.

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Address for correspondence: Sally A. Baylis, Division of Virology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK; fax: 44-1707-646-730; email: sbaylis@nibsc.ac.uk

Coordinated Response to SARS, Vancouver, Canada

Danuta M. Skowronski,* Martin Petric,* Patricia Daly,† Robert A. Parker,‡ Elizabeth Bryce,† Patrick W. Doyle,† Michael A. Noble,† Diane L. Roscoe,† Joan Tomblin,§ Tung C. Yang,¶ Mel Krajden,* David M. Patrick,* Babak Pourbohloul,* Swee Han Goh,* William R. Bowie,# Tim F. Booth, S. Aleina Tweed,* Thomas L. Perry,† Allison McGeer,†† and Robert C. Brunham***

Two Canadian urban areas received travelers with severe acute respiratory syndrome (SARS) before the World Health Organization issued its alert. By July 2003, Vancouver had identified 5 cases (4 imported); Toronto reported 247 cases (3 imported) and 43 deaths. Baseline preparedness for pandemic threats may account for the absence of sustained transmission and fewer cases of SARS in Vancouver.

In Canada, 2 urban areas received returning travelers infected with severe acute respiratory syndrome–associated coronavirus (SARS-CoV) from the original Hotel M cluster in Hong Kong. These travelers returned to Canada before the World Health Organization (WHO) issued its first global alert on March 12, 2003. One infected traveler from Hotel M returned to the greater Toronto area (GTA, population 4.7 million), Ontario; 2 returned to the Vancouver census metropolitan area (VCMA, population 2.0 million), British Columbia (BC). GTA, Ontario, is located in central Canada ≈4,000 km from VCMA, BC, which is the westernmost province of Canada. Control of SARS in both GTA and VCMA was by a national, publicly funded, but provincially administered healthcare system. Whereas GTA experienced sustained transmission, VCMA did not. Ultimately, GTA reported 247 patients with SARS and 43 related deaths; 3 cases were imported. VCMA identified 5 confirmed cases, 4 of which were imported (*1–5*). The experience with SARS in Vancouver highlights how a well-coordinated response of baseline precautions, reinforced through timely public health alerts and periodic infection control audits, can mitigate outbreaks due to emerging respiratory-borne pathogens.

*British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada; †Vancouver Coastal Health, Vancouver, British Columbia, Canada; ‡Fraser Health, New Westminster, British Columbia, Canada; §Royal Columbian Hospital, New Westminster, British Columbia, Canada; ¶St. Paul's Hospital, Vancouver, British Columbia, Canada; #University of British Columbia, Vancouver, British Columbia, Canada; **National Microbiology Laboratory, Winnipeg, Manitoba, Canada; and ††Mount Sinai Hospital, Toronto, Ontario, Canada

The Outbreak

Neutralization antibody titers to SARS-CoV among patients in VCMA are shown in Table 1. SARS-CoV was also confirmed in all but patient 1 by reverse transcription–polymerase chain reaction with multiple distinct primer sets applied to multiple specimens (*3,6–9*).

Patient 0 and patient 1 were a couple, who stayed on the 14th floor of Hotel M from February 20 to 24, 2003, and again from March 3 to 6. Both became ill on February 26 (Table 2). They returned to Canada on March 6 and went directly from the airport to their physician in Vancouver on March 7 (day 9 of illness). The husband (patient 0) was sent directly to the emergency room of a tertiary-care hospital (hospital A), arriving at 1:55 p.m. Within 15 minutes, full respiratory precautions were instituted. He was moved to a private room in the emergency room at 2:20 p.m. and transferred to a negative-pressure isolation room (NPIR) at 4:20 p.m. He was admitted into an NPIR of the intensive care unit (ICU) with full respiratory precautions at 6 a.m. on March 8 (Table 2).

His wife, patient 1, was recovering from mild illness, and no further follow-up was arranged. The couple had no other household contacts. Review confirmed that symptoms had not developed in any of the 148 hospital workers involved in patient 0's care by 10 days after his arrival at the hospital. The family physician had no detectable neutralizing antibody to SARS-CoV when tested at day 496.

Patient 2 of the VCMA had prolonged contact abroad with 2 family members in Hong Kong, who subsequently died from SARS. Although asymptomatic, she went to her physician in VCMA on March 26 because she was concerned about her exposure. Chest radiograph showed bilateral consolidation, and she was directed, masked, to hospital B, where she was admitted directly to an NPIR. She was transferred to the ICU of hospital C for assisted ventilation (Table 2). Neither of her 2 household contacts had detectable SARS-CoV antibody at day 215.

Patient 3 stayed at Amoy Gardens March 28–30 (*10*). Upon return, he remained self-isolated in the VCMA in the basement suite of his home with no contacts (household members were nevertheless quarantined, but they remained asymptomatic). Masked and short of breath, he sought treatment at hospital A on April 3. Initial chest radiograph was normal, but computed tomography scan showed widespread, patchy, ground-glass opacification of both lungs.

Table 1. SARS CoV antibody titers by microneutralization assay in persons with laboratory-confirmed SARS in VCMA*

Serum sample no.	Patient 0		Patient 1		Patient 2		Patient 3		Patient 4	
	Days after onset	Titer	Days after onset	Titer	Days after onset	Titer	Days after onset	Titer	Days after onset	Titer
1	10	1:32	16	1:128	4	<1:8	3	<1:8	11	<1:8
2	19	1:128	29	1:32	14	1:32	36	1:128	28	1:32
3	45	1:32	637	1:64	20	1:128	224	1:128	203	1:128
4					217	1:128	466	1:64	463	1:64
5					481	1:64				

*By number of days after symptom onset that serum was collected. SARS, severe acute respiratory syndrome; CoV, coronavirus; VCMA, Vancouver census metropolitan area.

He was admitted to hospital D directly to an NPIR (Table 2). His son, who drove him to hospital masked, had no detectable antibody to SARS-CoV at day 200.

Patient 4 of the VCMA was a nurse who cared for patient 2 at hospital B from March 29 to 30. At the time, patient 2 was receiving oxygen by mask and nebulization therapy. Patient 4 assisted patient 2 in using the toilet, which was flushed with lid raised in her presence. She followed guidelines in place at the time, but these did not include eye protection. Symptoms developed in the nurse on April 4. She went to hospital E on April 15, where she was admitted directly to an NPIR. Her only household contact remained asymptomatic. Neither he, nor a physician who examined her on April 11, had detectable SARS CoV antibody at 200 and 365 days, respectively.

All 5 patients with SARS in VCMA recovered fully. No additional unrecognized spread was evident. None of 442 staff members of hospitals A–E who participated in a voluntary serosurvey had detectable SARS-CoV antibody by microneutralization assay (details available from corresponding author, upon request).

Conclusions

Mathematical models for SARS, incorporating contact network theory, stress the importance of patient 0 in predicting the likelihood of an epidemic (*11*). This likelihood can be determined by the transmissibility of the agent, number of contacts of patient 0, and number of persons infected between patient 0 (the first patient infected) and intervention on the index patient (the first recognized case-patient). From this perspective, the circumstances of patient 0 in Vancouver compared to patient 0 in the Toronto, Canada, outbreak, merit closer examination.

Approximately 2,000 passengers land in Vancouver on direct flights from Hong Kong and mainland China every day compared with 500 on average to Toronto. As such, Vancouver is a potential gateway to North America for emerging pathogens from Asia. Because of this perceived risk, the BC Centre for Disease Control (BCCDC) had been increasing preparedness for pandemic threats for several years. An electronic distribution system was established to regularly disseminate communicable disease bulletins to healthcare facilities across the province. When a cluster of unexplained atypical pneumonia in China was reported almost simultaneously with reemergence of influenza A H5N1 in Hong Kong, BCCDC used this well-established communication network to issue an alert on February 20, 2003. The alert requested enhanced vigilance for severe influenzalike illness in returning travelers from mainland China or Hong Kong or among their close contacts. Alerts were repeated February 24, February 28, and March 12, 2003. Before patient 0's arrival, the emergency room at hospital A also participated in an infection control audit that emphasized that barrier precautions should be applied with all acute-onset respiratory infections. Patient 0 thus became the index patient in VCMA and was managed cautiously, even before WHO special alerts were issued. He sought treatment at the cusp of his peak infectious period at a tertiary-care hospital that had been repeatedly primed towards precaution. As a returned traveler, he was a first-generation case. He had no family contacts other than his wife, with whom he had traveled. Infection control precautions were implemented almost immediately upon his arrival at the hospital, limiting opportunities for spread.

When SARS arose in Ontario, a comparable agency to BCCDC did not exist. Responsibility for communicable disease control had shifted over the course of several years to local health boards, which created a decentralized system (*12*). Patient 0 in Toronto also stayed at Hotel M with her spouse from February 18 to 21. She returned to the GTA on February 23 to an apartment she shared with 5 family members (*5*,*13*). She died at home on March 5. During this period, she infected her 43-year-old son. This son became Toronto's index patient, a locally acquired, second-generation case (*5*,*13*). He went to a community hospital on March 7, the same day as Vancouver's patient 0, but was not recognized as a special threat. He was placed in general observation in the emergency room, where he remained for 18 hours and where he was given nebulized salbutamol. He was not placed in airborne isolation until he had been at the hospital for 21 hours; droplet and contact precautions were later begun on March 10 (*5*,*13*). By the time WHO issued its global alert, at least 14 persons in GTA had already become infected through 4 generations of spread: half within patient 0's family and the remainder among

healthcare contacts. Concern about severe illness in family members as they sought treatment at the hospital prompted an evening phone consultation on March 13 from an infection control practitioner in Toronto to the BCCDC in Vancouver. This call linked the separate Toronto and Vancouver cases to events in Asia and led to recognition that SARS had spread beyond that region. It also prompted WHO to issue a rare travel advisory on March 15 (*14*). Thereafter, awareness of precautions to be taken was enhanced everywhere, and further importations into Canada (Vancouver and Toronto) did not result in spread.

Ultimately, standard droplet and contact precautions proved an effective barrier to SARS except in the context of superspreading events such as aerosolizing procedures (*3*). Low inherent transmissibility, combined with the delay in peak infectivity until well into the course of

Table 2. Epidemiologic and clinical profile of patients with confirmed SARS, Vancouver*

Patient characteristics	Patient 0	Patient 1	Patient 2	Patient 3	Patient 4
Baseline characteristics					
Sex	Male	Female	Female	Male	Female
Age (y)	55	54	64	49	44
Medical condition	No	Diabetes	Hypertension	No	No
Epidemiologic characteristics					
Travel related	Yes	Yes	Yes	Yes	No
City of likely source of SARS	Hong Kong	Hong Kong	Hong Kong	Hong Kong	VCMA
Known contact with SARS	No	No	Yes	No	Yes
Likely date(s) of exposure, 2003	Feb 21	Feb 21	Mar 19	Mar 28–30	Mar 29 or Mar 30
Likely setting of exposure	Hotel M	Hotel M	Dinner party	Amoy Gardens	Hospital B
Date of return to Canada, 2003	Mar 6	Mar 6	Mar 20	Mar 30	NA
Clinical profile					
Symptoms and onset, 2003					
Malaise	Feb 26	Feb 26	Mar 24	No	Apr 4
Myalgia	No	Feb 28	Mar 24	Apr 1	Apr 10
Headache	Feb 28	Feb 28	Mar 27	Apr 1	Apr 4
Fever	Feb 28	Feb. 28	Mar 29	Apr 1	Apr 15
Chills	Feb 28	Feb 28	Mar 29	No	No
Chest discomfort	No	No	Mar 24	No	No
Cough	Mar 1	No	Mar 29	No	Apr 11
Shortness of breath	Mar 1	No	Mar 29	Apr 3	Apr 11
Nausea	No	No	Mar 27	No	No
Vomiting	No	No	No	No	No
Diarrhea	Mar 7	No	Mar 28	Apr 6	Apr 11
Hospitalized	Yes	No	Yes	Yes	Yes
Oxygen saturation (%) on room air at admission	45	NA	80	97; fell to 62 within 3 h	86
Aerosolized medication or nebulizer before isolation	No	No	No	No	No
Date of hospital admission	Mar 7	NA	Mar 28	Apr 3	Apr 15
No. days after symptom onset that patient was hospitalized	10		4	2	11
Date of final hospital discharge	Jun 12	NA	Apr 21	Apr 21	May 24
ICU	Yes	No	Yes	No	Yes
Date of ICU admission	Mar 8	NA	Apr 1	NA	Apr 15
Date of ICU discharge	May 13	NA	Apr 18	NA	Apr 24
Mechanical ventilation	Yes	No	Yes	No	No
Delay to implementation at hospital of:					
Respiratory precautions†	15 min‡	NA	Immediate§	Immediate§	7 min¶
Negative-pressure isolation	165 min‡	NA	Immediate§	Immediate§	11 min¶

*SARS, severe acute respiratory syndrome; VCMA, Vancouver census metropolitan area; NA, not applicable; ICU, intensive care unit; ER, emergency room; NPIR, negative-pressure isolation room.
†Defined as standard precautions (gloves, gown, eyewear) plus N95 mask and mask on patient when transported. Full respiratory precautions also include NPIR.
‡Arrived in triage March 7, 2003 1:55 p.m. By 2:10 p.m., admission sheet advises "full respiratory precautions" be taken. By 2:20 p.m. in single room in ER. Transferred to NPIR in ER at 4:40 p.m.
§Arrived at hospital masked and admitted directly into NPIR.
¶Arrived in ER on April 14, 2003, at 9:49 p.m. Identified as suspected SARS patient at 9:56 p.m. Masked and transferred to NPIR in ER at 10 p.m. Admitted to ICU NPIR on April 15.

serious illness, may explain why SARS was primarily a nosocomial infection and why so few countries experienced outbreaks (*3*). Patient 0 tests the baseline capacity of a system to respond to emerging threats before they are known or recognized. While favorable random chance may have played a role, Vancouver's response to SARS should not be dismissed on the basis of luck alone. Pasteur's edict that "chance favors only the prepared mind" may have modern relevance to the prepared healthcare system (*15*). The response to patient 0 in Vancouver highlights the importance of central coordination, baseline preparedness at the local level, and an efficient network of communication in mitigating outbreaks. Baseline preparedness should include barrier precautions in the care of all acute-onset respiratory infections. These should be reinforced through timely public health alerts and periodic infection control audits.

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Dr Skowronski is an epidemiologist at the BC Centre for Disease Control, responsible for surveillance, program and policy recommendations, and research related to respiratory-borne and vaccine-preventable diseases.

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Address for correspondence: Danuta M. Skowronski, BC Centre for Disease Control, 655 West 12th Ave, Vancouver, BC, Canada; fax: 604-660-0197; email: danuta.skowronski@bccdc.ca

Pathogen Transmission and Clinic Scheduling

John R. Hotchkiss,* David G. Strike,†‡ and Philip S. Crooke§

We developed a model of pathogen dissemination in the outpatient clinic that incorporates key kinetic aspects of the transmission process, as well as uncertainty regarding whether or not each incident patient is contagious. Assigning appointments late in the day to patients suspected of being infectious should decrease pathogen dissemination.

Pathogen dissemination within hospitals has been extensively analyzed (*1*). However, it has been the subject of far fewer investigations within the outpatient clinic. We developed a model of pathogen dissemination in the outpatient clinic and explored the anticipated effects of a system-based intervention (temporal segregation of suspected infectious and noninfectious patients) and an individual-based intervention (increased compliance with hand hygiene) on the risk that an uncontaminated patient will become contaminated during a clinic visit. An annotated copy of the model, as well as more detailed simulations and supporting material, may be obtained from the corresponding author.

The Model

We treat pathogen dissemination as a stochastic (chance-based) sequence of discrete encounters between incident patients (P) who are either infectious or noninfectious, a caregiver (C), and the environment (fomites, such as surfaces or waiting room magazines, [E]), each of which can be contaminated or uncontaminated. Four classes of encounter exist: 1) caregiver-patient, 2) caregiver-environment, 3) patient-environment, and 4) patient-patient. For each class of encounter, a user specifies a probability that a contaminated or infectious participant can transmit the pathogen to an uncontaminated participant (caregiver, environment [fomites], and surrounding patients). A contaminated caregiver can, in turn, contaminate subsequent patients (Figure 1). The contamination probabilities are not predicated on a specific mode of transmission but represent the gross probability that a contaminated or infectious participant will contaminate an uncontaminated participant during an encounter. With the exception of patient-to-patient transmission (see below) the contamination probabilities can be asymmetrical. Asymmetrical contamination probabilities allow consideration of droplet transmission; e.g., an infectious patient could cough on a caregiver, who then transfers the pathogen from his or her hands to the next patient.

An infectious patient can also contaminate the 4 patients surrounding him or her in the clinic queue (2 preceding and 2 following patients). The model does not incorporate patient-patient transmission by contaminated patients, given the relatively low frequency of intimate patient-to-patient physical contact in the clinic.

If contaminated, the caregiver may be decontaminated, e.g., by hand hygiene or pathogen attrition, with a specified probability after each patient visit. Similarly, a specified probability exists that the environment, if contaminated, will be decontaminated between visits. These 2 decontamination processes (for the caregiver and

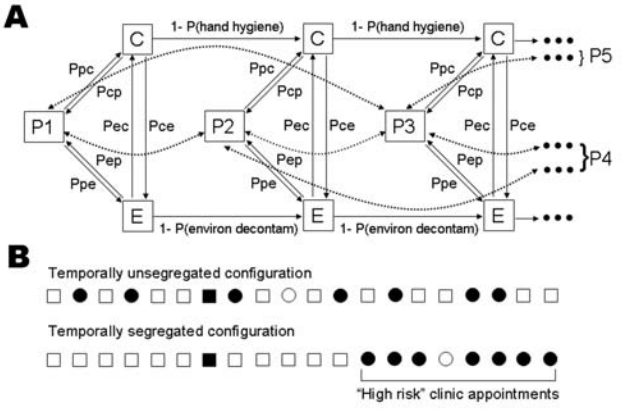

Figure 1. Schematic of model and segregation. A) Depiction of first 3 patient encounters. P1, P2, and P3, patients 1, 2, and 3; C, caregiver; E, environment. Arrows depict path (direction) of transmission if 1 participant in the interaction is infectious or contaminated. P(hand hygiene), probability that a contaminated caregiver will clear his or her contamination between patients; P(environ decontam), probability that a contaminated environment will be effectively decontaminated between patient visits. Direct patient-to-patient transmission is shown by dashed arrows. Because the model treats patient-to-patient transmission as a symmetrical process (the probability of transmission from patient to patient is identical regardless of which of the interacting patients is infectious), dashed arrows have 2 heads. B) Effects of different scheduling strategies. Solid circles, infectious high-risk patients; open circles, noninfectious high-risk patients; solid squares, infectious low-risk patients; open squares, noninfectious low-risk patients. Dots and arrows leading to P4 and P5 represent continuation of the chain of transmission. Ppc, probability of transmission from patient to caregiver; Pcp, probability of transmission from caregiver to patient; Pec, probability of transmission from environment to caregiver; Pce, probability of transmission from caregiver to environment.; Ppe, probability of transmission from patient to environment; Pep, probability of transmission from environment to patient.

*University of Pittsburgh, Pittsburgh, Pennsylvania, USA; †Regions Hospital, St. Paul, Minnesota, USA; ‡University of Minnesota, Minneapolis, Minnesota, USA; and §Vanderbilt University, Nashville, Tennessee, USA

the environment) are independent. Both the caregiver and the environment can be recontaminated after decontamination. The contamination probabilities for transmission from a contaminated environment to a patient or the caregiver are fixed (i.e., not a function of the number of preceding infectious persons).

The model generates a random number for each class of encounter between potentially contaminated or infectious and uncontaminated participants during each clinic appointment slot. If this random number is less than the contamination probability specific to the class of interaction being considered, the uncontaminated member becomes contaminated during the encounter.

The user specifies the population prevalence of the pathogen. A screening instrument for classifying patients as high or low risk for being infectious is assumed to exist. We assume that the screening instrument can be applied before the patient's clinic visit (at the time of appointment scheduling). The screening instrument could comprise a symptom inventory, knowledge of recent travel, household exposure, membership in a known high-risk group, or a combination of these or other elements. Such screening instruments exist for methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and severe acute respiratory syndrome (SARS) (*2*–*5*). The postulated instrument has a defined sensitivity and specificity; values that can be combined with the population prevalence of infectious patients to determine how many patients deemed at high risk or low risk are actually contagious.

Incident patients are assigned to appointment times following 1 of 2 protocols. In the baseline protocol, patients are randomly assigned appointments without regard to their risk status. In the segregated protocol, patients at high risk are assigned to appointments at the end of the clinic day (Figure 1). Clinics that segregate high-risk patients to later appointments might also adopt more stringent infection control strategies during the portion of the day populated by high-risk patients. Accordingly, the user can specify values for each contamination probability that differ between low-risk (early in the day) and high-risk (late in the day) clinic slots.

We modeled 1 day in a clinic in which 20 patients are seen by 1 caregiver. The model predicts the likelihood (risk) that a previously uncontaminated patient will become contaminated during his or her clinic visit. The model also predicts the risk that a patient who is classified as high-risk based on the screening instrument, but who is not infectious, will be contaminated in the segregated configuration.

Figure 2 illustrates the effect of temporally segregating patients deemed to be at high risk of being infectious to clinic appointments late in the clinic schedule (panel A) and the consequences of changes in caregiver hand-

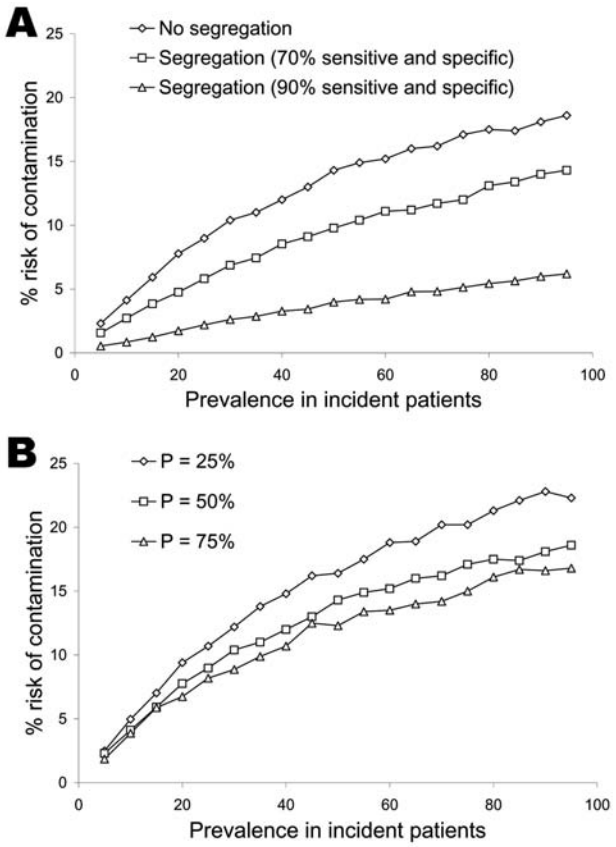

Figure 2. Risk that an uncontaminated patient will become contaminated during his or her clinic visit as a function of pathogen prevalence in incident patients and clinic infection-control practices. A) Predicted effects of temporally segregating patients at high risk of being infectious to appointments at the end of the clinic day, using a screening instrument that is either 70% sensitive and specific or 90% sensitive and specific. Transmission, hand hygiene, and environmental decontamination probabilities are as given in Table 1. B) Effects of varying levels of effective caregiver hand hygiene (25%, 50%, or 75%) on pathogen dissemination. All other inputs (probabilities of contamination) are identical to those in A. Each data point represents the mean of 2,000 simulations of a model day. An annotated copy of the model, as well as more detailed simulations and supporting material, may be obtained from the corresponding author.

hygiene compliance (panel B). Relevant pathogen-contamination probabilities were arbitrarily fixed at 20%, a level that is reasonable, and possibly conservative, for a wide range of pathogens (Table 1) (*6*–*14*). Temporal segregation substantially decreases the risk for pathogen dissemination, an effect that is at least comparable in magnitude to that arising from changes in the likelihood of effective hand hygiene within the clinically relevant range (*15*).

Table 2 addresses the ethical concern of the increase in contamination risk faced by noninfectious incident patients who are classified as high risk, using the same system inputs shown in Figure 2. The absolute risk for

Table 1. Interparticipant transmission and intraindividual transition probabilities used in simulations*

	Patient negative	Caregiver negative	Environment negative
Patient positive	0	$P_{PC} = 0.2$	$P_{PE} = 0.2$
Caregiver positive	$P_{CP} = 0.2$	P(hand hygiene) = 0.5	$P_{CE} = 0$
Environment positive	$P_{EP} = 0.2$	$P_{EC} = 0.2$	P(environ decont) = 0
Temporally adjacent patient positive	$P_{PP} = 0$	–	–

*P_{PC}, probability of transmission from patient to caregiver; P_{PE}, probability of transmission from patient to environment; P_{CP}, probability of transmission from caregiver to patient; P(hand hygiene), probability that a contaminated caregiver will clear his or her contamination between patients; P_{CE}, probability of transmission from caregiver to environment; P_{EP}, probability of transmission from environment to patient; P_{EC}, probability of transmission from environment to caregiver; P(environ decont), probability that contaminated environment will be decontaminated; P_{PP}, probability of transmission from patient to patient.

contamination faced by noninfectious but high-risk patients increases at all levels of prevalence and screening tool sensitivity and specificity; however, this increase does not exceed 6% (in the setting of a completely ineffective screening tool). More extensive analysis demonstrates that selectively deploying more aggressive infection-control practices to slots designated as high risk can lower the risk faced by noninfectious but nominally high-risk patients to below its value in the unsegregated configuration. These data are available from the corresponding author.

Conclusions

The results do not address direct patient-to-patient pathogen transmission; such transmission does not change the qualitative predictions of the model. The model does not incorporate either the potential for the environmental pathogen load to increase over time or differences in host susceptibility. The potential for cross-transmission of additional pathogens that have a higher prevalence in the high-risk group is also not addressed. For example, a population of patients deemed at high risk of having influenza or SARS might also have a higher prevalence of other transmissible pathogens, such as *Mycobacterium tuberculosis*.

Improved hand hygiene, barrier precautions, patient use of hand sanitizers or facemasks, and avoidance of environmental fomites can each diminish dissemination risk. However, these measures depend on individual behavior, rendering them susceptible to implementation failure. Temporal segregation adds a system-based layer of protection to such behavior-based interventions, providing an additional barrier to pathogen dissemination that is not critically dependent on individual compliance. When additional data addressing pathogen transmission within the clinic become available (currently such data are sparse), mathematical models could help guide the allocation of resources required to support system-based infection-control measures. Moreover, numerical experimentation could help inform the design of infection-control strategies for pathogens that have transmission dynamics that are not yet well characterized.

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Dr Hotchkiss is assistant professor of critical care medicine at the University of Pittsburgh School of Medicine. He has a long-standing interest in mathematical modeling; his more recent interests include dynamic systems analyses of infectious processes and pathogen dissemination.

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Table 2. Maximum absolute changes in contamination risk associated with temporal segregation

	Low-risk population		Noninfectious high-risk population	
Prevalence (%)	Maximum (%)	Minimum (%)	Maximum (%)	Minimum (%)
5	–2.1	–1	1	0.27
10	–4	–1.8	1.6	1.1
20	–6.8	–2.7	3.6	0
40	–10.7	–4.4	5.7	1

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Address for correspondence: John R. Hotchkiss, Department of Critical Care Medicine, University of Pittsburgh, 646 Scaife Hall, 3550 Terrace St, Pittsburgh, PA 15261, USA; fax: 412-647-8060; email: HotchkissJR@upmc.edu

Histoplasmosis Cluster, Golf Course, Canada

Heather Anderson,* Lance Honish,† Geoff Taylor,* Marcia Johnson,† Chrystyna Tovstiuk,† Anne Fanning,* Gregory Tyrrell,‡ Robert Rennie,§ Joy Jaipaul,† Crystal Sand,§ and Steven Probert†

We report a cluster of 4 cases of acute histoplasmosis (1 culture proven and 3 with positive serology, of which 2 were symptomatic) associated with exposure to soil during a golf course renovation. Patients in western Canada with compatible symptoms should be tested for histoplasmosis, regardless of their travel or exposure history.

Histoplasmosis is endemic in parts of the United States, South America, Southeast Asia, Africa, and Australia (*1*). Infection is almost always acquired by inhalation from an environmental source, most commonly disturbed earth or bird or bat droppings. Outbreaks and clusters of acute histoplasmosis from common sources are occasionally reported. The distribution of histoplasmosis has been largely described on the basis of these outbreak reports and histoplasmin skin-test surveys (*1–5*). In Canada, acute histoplasmosis occurs mainly in the central provinces with some evidence of infection in the Atlantic provinces and northern territories (*6–10*). Sporadic reports of cases from western Canada have been reported; however, local acquisition in these cases could not be verified (*11*,*12*). We report a cluster of cases of acute histoplasmosis among persons with no history of travel outside of Alberta during the incubation period for infection. This cluster is associated with exposure to disturbed soil during a golf course renovation.

The Outbreak

Case 1 (index case-patient): a 25-year-old woman with a history of headache, vomiting, fever, and cough sought treatment at a hospital. A chest radiograph showed diffuse nodular pulmonary infiltrates. Computed tomography showed numerous small nodules throughout both lungs. Thorascopic lung biopsy yielded necrotizing granulomata; stains did not show an organism. *Histoplasma* H and M bands were demonstrated in blood by immunodiffusion (ID) conducted by the National Center for Mycology (Edmonton), and culture of sputum and lung biopsy material yielded *Histoplasma capsulatum*. Itraconazole therapy was initiated. The patient previously resided in a village in Newfoundland but had relocated to Alberta 5 months before onset of illness. She was a maintenance worker at a golf course in suburban Edmonton and reported that several co-workers had experienced similar symptoms after the renovation of a fairway.

Clinical and exposure information was collected from a core group of 7 persons who worked with the index patient. Three additional probable or possible cases of histoplasmosis were identified and are described as follows.

Case 2: an 18-year-old man who was part of the golf course grounds maintenance crew that had worked on the renovation had fever, chills, headache, malaise, chest pain with deep inspiration, anorexia, and fatigue during the same period as the index case-patient, but did not seek medical attention. Blood for serologic testing was drawn 4 weeks after illness onset. The ID test result for *H. capsulatum* was positive for H and M bands, but a specimen was not submitted to the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, for complement fixation testing. A chest radiograph demonstrated no abnormalities.

Case 3: a 21-year-old man who also worked on the renovation project had fever, chills, nausea, headache, and malaise during the week after onset of the index patient's symptoms; his symptoms gradually resolved over 2 weeks. Serologic testing was conducted. *Histoplasma* ID was reported as negative, although 1 line of nonidentity was observed. A second sample drawn 6 weeks after illness onset was positive by ID. This specimen was submitted to CDC for further testing; there, ID exhibited both H and M bands and complement fixation testing found a titer of 1:8 for histoplasmin and >1:256 for whole yeast.

Case 4: a 37-year-old man who worked in the renovation of the golf course reported no illness during the period of interest. However, as in case 3, despite negative *Histoplasma* ID results on serologic testing, 1 line of nonidentity was observed. A serum sample drawn from this individual 6 weeks after the index patient's onset of illness was tested at CDC. ID for *H. capsulatum* was positive for M band only, and complement fixation testing was reported as positive for histoplasmin (titer 1:4) and whole yeast (1:64).

Of the remaining 4 co-workers screened for clinical and exposure information, 1 reported onset of respiratory illness at the same time as the case-patients although all had negative *H. capsulatum* serology as conducted by the National Center for Mycology. Other golf course employees and contract workers were advised to be tested. Of the

*University of Alberta, Edmonton, Alberta, Canada; †Capital Health, Edmonton, Alberta, Canada; ‡Alberta Provincial Laboratory for Public Health, Edmonton, Alberta, Canada; and §National Center for Mycology, Edmonton, Alberta, Canada

70 staff, 51 provided a serum sample for testing. No additional cases were identified.

Exposure to disturbed soil during golf course renovation was investigated as a possible source of the cluster. Renovations included sod and tree removal, grading of existing soil (no soil was imported), and sod replacement. Workers reported that dust was generated on the course during these activities. Replacement sod came from British Columbia. The approximate incubation periods (i.e., period between the end of the renovation and date of illness onset) for the 3 symptomatic case patients (Cases 1–3) were 6, 6, and 12 days, respectively; however, these are likely underestimates, as the exact date of exposure during the 2-week renovation project is unknown. No accumulated bird or bat droppings within facility buildings nor bird or bat roosting sites on the golf course were evident. Soil samples were tested for *H. capsulatum*, but all were negative.

Conclusions

In this cluster of 4 cases, the index case-patient unequivocally experienced acute pulmonary histoplasmosis. Two other persons epidemiologically associated with the index case had compatible symptoms and positive serologic results, and thus can be considered probable case-patients. The fourth patient, although epidemiologically associated with the others, was never symptomatic. While serology from this patient was weakly positive, false-positive serologic results or remote infection cannot be excluded; thus, he is considered a possible case-patient. Together, these cases establish the possibility of local acquisition of histoplasmosis in northern Alberta with a probable micro-focus of soil contamination.

As part of this investigation, an unsuccessful attempt was made to identify soil contaminated with *Histoplasma* from the implicated golf course. Nevertheless, on the basis of the patients' epidemiologic association and development of symptoms within the incubation time for this infection (*13*), local soil exposure was the likely source of their infections. A less likely source is the replacement sod imported from British Columbia; however, the laying of sod was not associated with dirt aerosolization and histoplasmosis is not endemic in British Columbia.

The environmental reservoir for *H. capsulatum* is soil with an acidic pH, some degree of moisture, and moderate temperature. Bird and bat droppings are thought to provide nutrients for *H. capsulatum* sporulation. Transmission does not occur directly from animals or person-to-person. As inhalation is the nearly universal route of acquisition of this agent, environmental risk factors usually involve the disruption of soil or other infected material (*14*,*15*). Aerosolized *H. capsulatum* can be generated during excavation, construction, or demolition of work areas, cleaning of sites with accumulated bird or bat droppings, and cave treks. Most major urban outbreaks have been associated with earth disruption. Two separate outbreaks in Mason City, Iowa, were related to bulldozing a park and the later removal of trees (*15*). Data collected in Indiana in the late 1970s and early 1980s identified 2 outbreaks linked to the construction of a tennis complex and excavation for a swimming pool, respectively (*15*).

This investigation demonstrates the use of acute disease clusters in defining the boundaries of the geographic distribution of histoplasmosis. Before this episode, histoplasmosis has not been considered endemic in northern Alberta; milder, self-resolving clinical cases may have gone unrecognized. Clinicians in western Canada should consider testing for histoplasmosis in clinically compatible patients regardless of their travel or exposure history.

Dr Anderson is a graduate of the University of Alberta Faculty of Medicine and Dentistry and is currently a resident in Internal Medicine at the University of Alberta.

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Address for correspondence: Geoff Taylor, 2E4.14 Walter Mackenzie Center, University of Alberta, Edmonton, Alberta, Canada, T6G 2B7; fax: 780-407-7137; email: geoff.taylor@ualberta.ca

Neutralizing Antibodies in Survivors of Sin Nombre and Andes Hantavirus Infection

Francisca Valdivieso,* Pablo Vial,* Marcela Ferres,† Chunyan Ye,‡ Diane Goade,‡ Analia Cuiza,* and Brian Hjelle‡§

We evaluated titers of homotypic and heterotypic neutralizing antibodies (NAbs) to Andes and Sin Nombre hantaviruses in plasma samples from 20 patients from Chile and the United States. All but 1 patient had high titers of NAb. None of the plasma samples showed high titers against the heterologous virus.

Hantavirus cardiopulmonary syndrome (HCPS), an emergent disease caused by New World hantaviruses, is associated with case-fatality ratios of 30% to 50%. Sin Nombre virus (SNV) and Andes virus are well-characterized hantavirus serotypes responsible for this disease in the southwestern United States and in the south cone of the Americas (Argentina, Brazil, and Chile), respectively (*1*). Since their recognition in 1993, they have caused hundreds of cases, many of them appearing in seasonal outbreaks. Other types of hantaviruses have been identified in the Americas during the past decade, causing diseases with variable severity. All of them are associated with different rodent hosts of the subfamily *Sigmodontinae*, family *Muridae*, and the distribution of each virus parallels that of the host (*2*).

No specific treatment for HCPS exists. Ribavirin, the only approved antiviral agent that is effective against hantaviruses in vitro (*3*), has shown efficacy in treating hemorrhagic fever with renal syndrome, a related disease that is caused by hantaviruses indigenous to the Old World (*4*). However, technical difficulties prevented a trial that was designed to evaluate the efficacy of ribavirin in treating HCPS from being completed (*5*).

*Universidad del Desarrollo, Santiago, Chile; †Pontifica Universidad Catolica de Chile, Santiago, Chile; ‡University of New Mexico Health Sciences Center, Albuquerque, New Mexico, USA; and §TriCore Reference Laboratory, Albuquerque, New Mexico USA

Some evidence shows that neutralizing antibody (NAb) can affect the course of HCPS. Animal studies have shown that NAb confers passive protection from severe disease by Andes virus. Specifically, passive transfer of serum with high NAb titers from rhesus macaques vaccinated against Andes virus protected 100% of Syrian hamsters from lethal disease, even when administered 4–5 days after challenge with Andes virus (*6*). In humans, a high NAb titer on hospital admission is correlated with less severe HCPS (*7*)

Administering convalescent-phase plasma with a high NAb titer could be therapeutic in HCPS, as it is in other hemorrhagic fevers (*8*). In survivors of Sin Nombre infection, high titers of serum NAb could still be detected years after recovery, with no evidence of residual viral RNA in the plasma (*9*).

The severity of HCPS, the absence of effective treatment, its appearance in outbreaks and in case-clusters, and the potential use of hantaviruses as bioweapons have stimulated work toward hantavirus vaccine development. At present, an inactivated Hantaan virus vaccine is in use for persons at high risk for exposure to Old World hantaviruses, but its efficacy has recently been questioned (*10*). A DNA vaccine expressing the G1 and G2 glycoproteins encoded by the Hantaan virus M segment conferred sterilizing cross-protection against the other Old World hantaviruses, Seoul, Dobrava, and Puumala, in hamsters (*11*). For New World hantaviruses, in the hamster model for Andes disease, prior infection with widely disparate species conferred varying levels of cross-protection (*12*,*13*). Although these selected studies suggest some cross-protection among different hantavirus species, the considerable antigenic variation among members of the genus *Hantavirus* suggests that a monovalent vaccine will not likely confer sufficient protection for all of the pathogenic hantaviruses (*14*).

The persistence of NAb in plasma of survivors of Andes virus and SNV infections, as well as the in vitro cross-neutralization capacity of these NAbs against the heterotypic hantavirus, could have implications for use of convalescent-phase plasma to treat HCPS. For vaccine development, an evaluation of the duration of persistence of NAb and their cross-neutralization activities across different serotypes of hantaviruses would shed light upon the probability of obtaining satisfactory cross-protection among candidate vaccines against New World hantaviruses.

The Study

We studied 20 serum samples from survivors of confirmed hantavirus infection, 11 from Chilean patients and 9 from patients in the southwestern United States. Samples were collected from 8 months to 11 years after the patient was hospitalized with HCPS. The neutralizing titer was

measured for each sample against SNV and Andes virus by a focus-reduction neutralization assay in Vero E6 cells, as described previously (*7*). In brief, serial 2-fold dilutions of heat-inactivated patient plasma samples were made, from 1:100 to 1:1,600, and were mixed with equal volume of ≈50–100 focus-forming units per milliliter SNV (isolate SN77734, titer 2×10^6/mL) or Andes virus (Chilean strain of human origin, isolate CHI-7913) and incubated at 37° for 1 hour (*15*). The mixture was then used to infect a confluent monolayer of Vero E6 cells (ATCC CRL 1586) in duplicate wells of a 48-well dish, with a 1.2% methylcellulose overlay in the medium to confine the virus to the foci. After incubation for 1 week, viral foci were detected with polyclonal rabbit anti-N antibody followed by peroxidase-conjugated goat anti-rabbit immunoglobulin G. Foci were enumerated under an inverted light microscope. NAb titers were defined as the reciprocal of the highest serum dilution that resulted in an 80% reduction in the number of foci compared to virus controls in duplicate assays.

The endpoint plasma NAb titers against Andes virus and SNV from Chilean and North American survivors of hantavirus infection are shown in the Table. All Chilean patients had detectable plasma NAb against Andes virus, with titers ≥1:400 in all but 1 patient. In contrast, 9 of the 11 samples failed to show NAb titers ≥1:100 against SNV, while the other 2 neutralized SNV only at low titers. Similarly, all North American patients had plasma NAb against SNV at titers ≥400, and only 1 showed some neutralization against Andes virus, at low titer. No relationship was seen between the endpoint NAb titers against the homotypic virus and time elapsed from acute disease in either Chilean or North American patients, nor did a particularly high homotypic titer predict that neutralizing activity would be present against the heterologous virus.

Table. Neutralizing antibody (NAb) titers against Andes virus (AND) and Sin Nombre virus (SNV) in survivors of hantavirus infection from Chile and the United States.

Patient	Origin	Years after infection	AND NAb titer	SNV NAb titer
1	Chile	3	>1:1,600	1:100
2	Chile	4	1:400	<1:100
3	Chile	7	>1:1,600	<1:100
4	Chile	0.7	1:400	<1:100
5	Chile	4	1:400	<1:100
6	Chile	1	1:400	<1:100
7	Chile	4	1:800	1:100
8	Chile	3	1:200	<1:100
9	Chile	4	>1:1,600	<1:100
10	Chile	7	1:400	<1:100
11	Chile	1	1:400	<1:100
12	USA	1	<1:100	1:800
13	USA	3	<1:100	1:400
14	USA	4	<1:100	1:400
15	USA	4	1:100	>1:1,600
16	USA	3	<1:100	1:400
17	USA	5	<1:100	>1:1,600
18	USA	11	<1:100	1:400
19	USA	6	<1:100	1:800
20	USA	4	<1:100	>1:1,600

Conclusions

In survivors of hantavirus disease who reside in Chile or the United States, we found high titers of plasma NAb against the type of hantavirus that is prevalent in the patient's own region, while substantial titers against the heterologous agent of HCPS were absent. In this small group of participants, NAb titers did not show any readily detectable decline with time elapsed after infection; titers as high as 1:1,600 could be detected 11 years after illness. These results suggest that plasma from patients who survive hantavirus infection is a potential source of NAb and could be used as a therapeutic alternative for patients with acute disease or as a prophylactic intervention for persons who may have been exposed to the virus. The absence of in vitro cross-neutralization makes the alternative of clinically effective cross-protection less likely and discourages the use of convalescent-phase sera to treat patients whose geographic origin is different from that of the plasma donor. Our results suggest that a monovalent vaccine would not elicit protection against different types of hantavirus, even when the viruses are phylogenetically as similar as SNV and Andes virus. The positive results of cross-protection studies in hamster models should be interpreted cautiously, since experimental infection in those studies would tend to favor unusually brisk immune responses that go well beyond eliciting NAb and likely include potent cell-mediated or innate immune responses that cannot be mimicked with passive immunization (*12*). Similarly, some component of the cross- protective efficacy observed with genetic immunizations with hantavirus envelope genes may ultimately be related to T-cell immunity (*13*). From this perspective, either multivalent or region-specific vaccines may have to be developed to protect persons at high risk from this new, relatively infrequent, but still highly lethal disease.

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Dr Valdivieso is an assistant professor of microbiology at the Universidad del Desarrollo, Santiago, Chile. Her research interests include the epidemiology, pathogenesis, and treatment of hantavirus infections.

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Address for correspondence: Brian Hjelle, Infectious Diseases and Inflammation Program, Department of Pathology, University of New Mexico Health Sciences Center, MSC08 4640, 1 University of New Mexico, Albuquerque, NM 87131, USA; fax: 505-272-4401; email: bhjelle@salud.unm.edu

New Route of Importation of *Mycobacterium tuberculosis* Beijing Genotype

To the Editor: *Mycobacterium tuberculosis* (MTB) Beijing genotype is spread throughout the world, and the highest prevalence has been detected in Asia and Eurasia. In western Europe, the prevalence of Beijing strains is relatively low (*1*–*3*), and cases in immigrants with Beijing strains are mainly Asian in origin.

In Spain, the last few years have been a key period for infectious diseases because of the sharp increase in the immigrant population. The percentage of tuberculosis (TB) cases in immigrants in our area has increased from 6.7% (75/1,123) in 1997–1999 to 29.4% in 2002–2003 (184/625). Our study had 2 aims: 1) to determine the prevalence of Beijing strains in Madrid and thus establish a reference for this genetic family before the impact of nonnative TB cases is experienced, and 2) to check whether the general parameters found for Beijing strains in Europe are valid for Spain or whether specific features can be identified.

All the MTB isolates (n = 510; ≈30% of all MTB cases in Madrid), obtained from patients January 2001–December 2002 from 2 tertiary teaching hospitals in Madrid (population 1,383,790), were genotyped by IS*6110* restriction fragment length polymorphism (RFLP) and spoligotyping. The distribution of nationalities of the patients from the studied population did not differ from the distribution in Madrid as a whole; therefore, no selection bias is expected.

Beijing strains were identified in 8 case-patients (1.6%). Spoligotypes corresponded to the Beijing standard patterns (Figure A). The RFLP types showed >13 bands (Figure B). All isolates were susceptible to anti-TB drugs (except 1 streptomycin-resistant strain).

Six of 8 patients infected with Beijing strains were immigrants; 4 were from South America (3 from Peru, with no epidemiologic links between them, and 1 from Ecuador), 1 was from South Africa, and 1 was from Asia. The remaining 2 patients were indigenous to Spain. Patients from Latin America had an increased prevalence of the Beijing genotype (3.8%) compared to the Spanish patients (0.4%).

Although Asia has been the source of importation of Beijing strains in the Netherlands, Denmark, and Italy (*1*–*3*), our data showed a potential new South American source for imported Beijing strains in Europe. Most immigrants in Madrid come from South America (and make up 71.8% of the total immigrant population, excluding western Europeans), and 59.3% of all South Americans come from Peru and Ecuador. Therefore, this new route of importation will likely have an impact. A previous report from Spain (*4*) described the isolation of Beijing strains from Peruvian patients, which suggests that this finding is not recent or casual. Few studies have offered genotyping data of Beijing strains in Peru and Ecuador.

Despite the low prevalence of Beijing strains in our area, we found 2 patients infected by the same strain (Figure), which suggests transmission between them. Notably, this recent transmission event involved a case in an immigrant patient (Ecuadorian, patient 2) and an indigenous patient (patient 5) (Figure). Specimens from the Ecuadorian patient were positive for acid-fast bacilli; his TB symptoms appeared 2 months before his arrival in Spain, and he began anti-TB therapy 7 months after arrival. Studies focused on the analysis of recent transmission of strains between those born in other countries and the autochthonous population in Europe generally conclude that the transfer of strains between both groups is low or moderate (*5*–*7*). In Madrid, recent

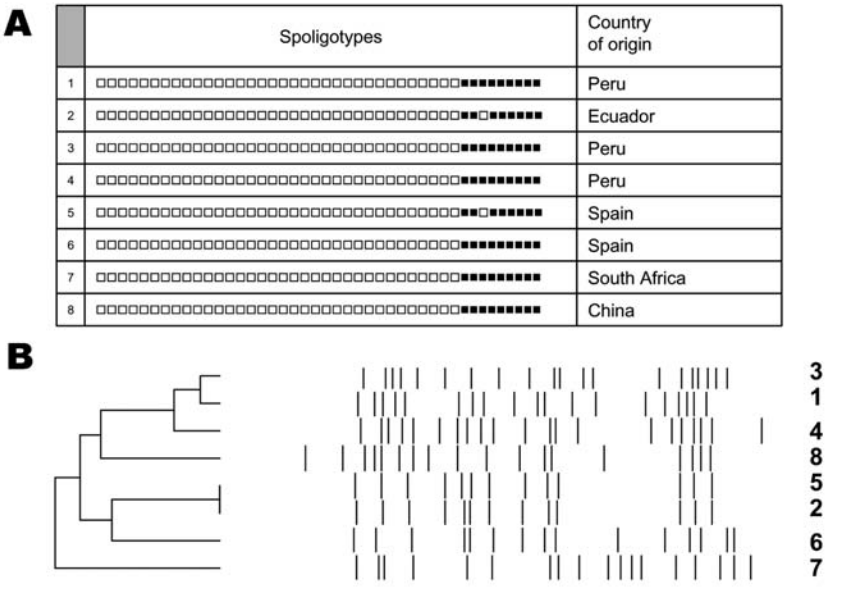

Figure. A) Spoligotypes from *Mycobacterium tuberculosis* (MTB) clinical isolates. Black boxes indicate hybridization with the corresponding spacer in the directed repeats region, and white boxes indicate the absence of hybridization. The country of origin and identification number for each patient are indicated. B) Similarity dendrogram of restriction fragment length polymorphism types obtained with MTB clinical isolates. Numbers on the right correspond to patient identification numbers.

transmission between the immigrant and autochthonous population has been frequently detected (*8*), likely indicating a fairly high degree of social permeability between the 2 groups. The transmission of Beijing MTB from a person born in another country to a person born in Spain reported here seems to follow this trend and raises concern about a potential spread of Beijing MTB to the autochthonous population.

The isolate from the other Spanish patient (patient 6) shared the genotype of the strain described earlier in the spread of Beijing genotype in Gran Canaria (*9*); a Liberian was the first case-patient. The patient from Spain in our report had been imprisoned on that island before her arrival in Madrid. These data suggest another way Beijing genotype can be imported into Spain, which is different from the South American route.

In summary, we describe TB patients with Beijing genotype strains in Madrid among patients from South America. This geographic origin differs from the predominant Asian origin reported for TB cases in other European countries caused by the Beijing genotype. Our findings suggest an alternate route of transmission between South America and Europe for the Beijing genotype. Furthermore, the recent occurrence of this genotype in a TB patient from Spain, who shared an RFLP type with a South American patient, suggests further transmission of these strains into the local community. Longitudinal studies should monitor the potential impact and establishment of these strains after their introduction.

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Darío García de Viedma,* Fernando Chaves,† and Jesús Iñigo,‡ for the Tuberculosis Molecular Epidemiology Study Group[1]

*Hospital Gregorio Marañón, Madrid, Spain; †Hospital Doce de Octubre, Madrid, Spain; and ‡Consejería de Sanidad, Madrid, Spain

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[1]Tuberculosis Molecular Epidemiology Study group (in alphabetical order): Sandra Andrés, Araceli Arce, Emilio Bouza, Fernando Chaves, Darío García de Viedma, Jesús Iñigo, Elia Palenque, Elena Rodríguez, M. Jesús Ruiz Serrano.

Address for correspondence: Darío García de Viedma, Servicio de Microbiología y Enfermedades Infecciosas, Hospital Gregorio Marañón, C/ Dr Esquerdo 46, 28007 Madrid; fax: 34-91-504-4906; email: dgviedma@microb.net

H5N1 Avian Influenza, Kampot Province, Cambodia

To the Editor: As a resident in pediatrics with an interest in infectious diseases, I was fascinated by the range and scope of conditions I saw in May and June of 2005 in local children in Kampot Province, a mainly rural area in southeastern Cambodia. This province, near the Vietnamese border, is the epicenter of H5N1 avian influenza in Cambodia, with all 4 known human cases of the disease. All of these documented human cases have been fatal.

While the World Health Organization and the Cambodian Ministry of Health have engaged in public education, the village-level response to a pathogen of potential global importance is evolving. As part of my work in Cambodia, I made numerous information-gathering visits to villages in Kampot Province. Though village elders and health workers had often heard of the "bird flu," most of the villagers I spoke to had not. Many persons did not know whom to contact should their chickens

or other birds die. Those who knew about H5N1 influenza told me that, without adequate compensation for culling flocks, little incentive would exist to report bird deaths. In a typical village, chickens, ducks, and pigs intermingled with each other and with humans underneath or around homes on stilts. General knowledge of infection control practices among villagers was minimal.

The dissemination of information into a rural, agricultural society such as that in the southeast of Cambodia is a difficult task. Many rural inhabitants do not have televisions or radios and may infrequently travel to larger towns. Health workers from international groups, nongovernmental organizations, and the government are often required to travel on foot or motorbike through fields and forests to reach and educate the population. Government health workers lack the personnel and resources to adequately identify and investigate potential cases, and Cambodia has substantially fewer microbiology laboratories than do neighboring Thailand and Vietnam.

Should a pandemic of avian influenza occur, it will almost certainly originate in Southeast Asia. Cambodian and international health organizations have recognized the country's potential key role in propagation of an impending pandemic agent. However, because of its history and current economic state, Cambodia is less able to respond to the avian influenza threat than its neighbors. In recognition of this fact, the World Health Organization and the Cambodian Ministry of Health have stated that the prevention, control, and identification of avian influenza are national priorities. Additionally, international funds have been flowing into Cambodia to assist with avian H5N1 influenza surveillance and case investigation. Much work remains to be done; we hope that by combining international resources and policy with domestic expertise and effort, Cambodia will mount a successful response against this emerging threat.

Shaun K. Morris*

*University of Toronto, Toronto, Ontario, Canada

Address for correspondence: Shaun K. Morris, Department of Pediatrics, The Hospital for Sick Children, The University of Toronto, 555 University Ave, Toronto, Ontario M5G1X8, Canada; email: shaun.morris@utoronto.ca

Helicobacter pylori and Immunocompromised Children

To the Editor: *Helicobacter pylori* has been classified as a carcinogenic pathogen. Its prevalence is high in developing countries. Apart from the known gastrointestinal pathologic changes caused by this organism, reports on the association between *H. pylori* infection and extragastrointestinal diseases have been increasing. Although impaired host immunity should be associated with a high prevalence of this infection, a definitive relationship has not been established. We conducted a cross-sectional study to determine the prevalence of *H. pylori* infection in immunocompromised Thai children.

The study was reviewed and approved by the research ethic committee of Chiang Mai University. From 2003 to 2004, a total of 60 children <18 years of age, who received corticosteroids, immunosuppressive drugs, or both, were enrolled consecutively into this study. Patients who had taken proton pump inhibitors and antimicrobial drugs 2 weeks before the study began were excluded. Stool specimens were collected and immediately stored at –20°C before analysis with the *H. pylori* stool antigen test (Meridian Bioscience Inc., Cincinnati, OH, USA). Although no study has validated this test in Thai children, most studies report its high sensitivity and specificity (>90%) (*1*).

The children enrolled in the study had a mean age of 7.9 years (range 0.5–16.6) and most were receiving both corticosteroids and chemotherapy (n = 36). Fourteen patients were being treated exclusively with corticosteroids, and 10 patients were receiving only chemotherapy. A total of 17.4% of the children <5 years of age had *H. pylori* infection, and the overall prevalence was 20%. Although we observed a relatively high prevalence of infection in patients with malignancy, particularly leukemia, the trend did not reach statistical significance (Table).

In contrast to previous studies that reported a low prevalence of infection with *H. pylori* in patients with AIDS (*2*) and leukemia (*3*), we demonstrated

Table. *Helicobacter pylori* stool antigen test results in immunocompromised children and primary diagnosis*

Primary diagnosis	*H. pylori* stool antigen test	
	No. positive	No. negative
Malignancy		
Leukemia	8	21
Lymphoma	2	3
Neuroblastoma	0	7
Retinoblastoma	0	2
Nonmalignancy		
Nephrotic syndrome	1	8
SLE	0	6
Chronic renal failure	1	1

*SLE, systemic lupus erythematosus.

that its prevalence in immunocompromised Thai children (20%) was higher than that previously reported in a healthy Thai population (17.5%) (*4*) and in those with recurrent abdominal pain (11.3%) (*5*). The prevalence in children <5 years of age was high compared with that reported from Perez-Perez et al. (*17.4% vs. 5%*) (*4*). Although unintentional eradication of *H. pylori* after multiple courses of antimicrobial drugs in such patients could explain the low prevalence in some studies, commonly prescribed antimicrobial drugs without antisecretory agents may be unable to cure the infection.

The major limitations of this preliminary study were the use of different diagnostic methods in the various studies and the lack of healthy controls. Thus, a well-designed case-control study is needed. However, the prevalence of infection with *H. pylori* in the immunocompromised children was high, and these patients appear to be more susceptible to this infection in early life.

Prakaimuk Nutpho*
and Nuthapong Ukarapol*
*Chiang Mai University, Chiang Mai, Thailand

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Address for correspondence: Nuthapong Ukarapol, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; fax: 66-53-946-461; email: nukarapo@chiangmai.ac.th

Community Case of Methicillin-resistant *Staphylococcus aureus* Infection

To the Editor: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is an emerging infectious disease worldwide and is increasingly reported in Asia (*1*). We describe a community case of invasive MRSA infection, which appeared as bacteremia and pneumonia; CA-MRSA was initially suspected, and eventually the patient was treated successfully with ampicillin/sulbactam.

A 52-year-old man with chronic eczema was admitted to the Prince of Wales Hospital, Hong Kong, with fever and chills. Before admission, he had been treated for infected eczematous lesions for several weeks with oral ampicillin, cloxacillin, and cefazolin. He had no history of hospitalization in the past 10 years, and none of his family members were healthcare workers. Examination showed an oral temperature of 40°C, blood pressure 95/55 mm Hg, and no audible murmur. Cellulitis in the left leg complicated his eczematous skin lesions. Chest radiograph showed right-middle-zone pneumonia. Neutrophilia (leukocytes 15.5×10^9/L, neutrophils 86%), thrombocytopenia (platelets 55×10^9/L), prolonged activated partial thromboplastin time (43.6 s), and elevated bilirubin level (31 μmol/L) were observed. Two initial blood cultures grew gram-positive cocci in clusters, identified as *S. aureus* by positive results for catalase and slide/tube coagulase and a negative result for ornithine decarboxylase. Intravenous cloxacillin (2 g every 6 h) was given on days 2–5. Antimicrobial drug susceptibility testing was performed by the disk-diffusion method (1 μg oxacillin/disk, Mueller-Hinton agar, 2% NaCl), followed by MIC determination with the agar dilution method in accordance with NCCLS (former National Committee for Clinical Laboratory Standards, now Clinical and Laboratory Standards Institute) recommendations (*2*). One blood isolate was identified as methicillin-resistant *S. aureus* (MRSA), with an oxacillin MIC 4 μg/mL. The other isolate was identified as methicillin-sensitive *S. aureus* (MSSA), with an oxacillin MIC of 0.5 μg/mL. In view of a possible CA-MRSA infection (which could have been β-lactam–resistant), cloxacillin was substituted with intravenous vancomycin plus rifampin on day 5.

However, the patient's condition progressively deteriorated from day 2 to day 10 with persistent fever, chills, hypotension, and hemoptysis. A repeated chest radiograph showed small lung cavities with fluid, and a thoracic computed tomographic scan confirmed multiple lung abscesses. Results of an initial transthoracic echocardiograph were normal, but a subsequent transesophageal echocardiograph demonstrated tricuspid valve vegetation.

The MRSA isolate was susceptible to gentamicin, cotrimoxazole, erythromycin, ciprofloxacin, clindamycin, fusidic acid, tetracycline, chloramphenicol, vancomycin, and rifampin; a different pattern of multidrug-resistant

Table. Comparison between methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*

	Strain and major resistance mechanism*			
	MSSA/penicillinase production	BORSA/novel methicillinase ± penicillinase hyperproduction	CA-MRSA/PBP alteration	HA-MRSA/PBP alteration
PBP2a detection (e.g., latex-agglutination method)	–	–	+	+
*mec*A gene detection (e.g., PCR method)	–	–	+ (SCC*mec* IVa)	+
PVL gene detection (PCR method)	Infrequent (<5%)	Data limited	Frequent (>66%–100%)	Infrequent (<5%)
Coresistance to non–β-lactam antimicrobial drugs	±	±	+	+++
Usual antimicrobial drugs to which MSSA is susceptible	PRP (e.g., cloxacillin), β-lactam/β-lactamase–inhibitor combinations (e.g., ampicillin/sulbactam); linezolid, vancomycin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, fluoroquinolones, rifampin, gentamicin, fusidic acid, tetracyclines	PRP (e.g., cloxacillin), β-lactam/β-lactamase–inhibitor combinations (e.g., ampicillin/sulbactam), other drugs to which MSSA is potentially susceptible	Vancomycin, linezolid, rifampin, gentamicin, trimethoprim-sulfamethoxazole, fusidic acid, tetracyclines, fluoroquinolone, clindamycin†	Vancomycin, linezolid; ± fusidic acid, rifampin, gentamicin, trimethoprim-sulfamethoxazole, fluoroquinolones‡

*MSSA, methicillin-susceptible *Staphylococcus aureus*; BORSA, borderline oxacillin-resistant *S. aureus*; MRSA, methicillin-resistant *S. aureus*; CA-MRSA, community-associated MRSA; HA-MRSA, hospital-associated MRSA; PBP, penicillin-binding protein; PCR, polymerase chain reaction; PVL, Panton-Valentine leukocidin; PRP, penicillinase-resistant penicillins; +, positive; –, negative. ±, occasionally present; +++, usually present.
†Concern over inducible clindamycin resistance; also, macrolide resistance is common.
‡Fluoroquinolone resistance increasing.

MRSA isolates from that usually found in our facility (*2*,*3*). The isolate was also susceptible to ampicillin/sulbactam, with an equivalent breakpoint MIC <8/4 μg/mL by disk testing (*2*). Latex detection for PBP2a (Slidex MRSA-Detection, bioMérieux, Marcy l'Etoile, France) and polymerase chain reaction (PCR) detection for *mec*A were both negative, predicting nonresistance to oxacillin (*2*–*5*). A nitrocefin-disk test was positive for β-lactamases, and a 4-fold reduction in MIC was demonstrated in the presence of sulbactam (*6*). Panton-Valentine leukocidin (PVL) gene locus was not detected (*1*). Community-acquired BORSA (borderline oxacillin-resistant *S. aureus*), infective endocarditis, and lung abscesses were diagnosed. Intravenous ampicillin/sulbactam (3 g every 6 h) was given on day 10 with rifampin; vancomycin treatment was stopped. Defervescence occurred 3 days later, subsequent blood cultures became sterile, and radiographic changes gradually resolved. Ampicillin/sulbactam was given for 6 weeks without complication.

As this case suggests, BORSA can sometimes be confused with CA-MRSA because of similar clinical signs and symptoms and overlapping oxacillin MICs (2–8 μg/mL and 4–64 μg/mL, respectively) (*1*,*4*,*6*). Both pathogens can appear as community-acquired infections and may be related to previous antimicrobial drug usage (*6*,*7*). Although CA-MRSA has been associated with soft tissue infections and necrotizing pneumonia (*7*,*8*), MSSA or BORSA strains can also cause these diseases. Thus, in view of potentially different treatment options, when MRSA isolates (e.g., oxacillin MICs ≥4–8 μg/mL) are associated with community-acquired, serious infections (e.g., blood isolates) and are not multidrug resistant, one can consider *mec*A (or PBP2a) testing to delineate the resistance mechanism (Table). If *mec*A is present, further testing for PVL gene locus with or without staphylococcal chromosomal cassette *mec* (*SCCmec*) type IV can be performed to diagnose CA-MRSA; if *mec*A is not detected, further testing for BORSA may be indicated, and β-lactam therapy should be evaluated individually. If these pathogens are not differentiated and all are treated as CA-MRSA, a non–β-lactam antimicrobial drug, such as vancomycin, will be used (*1*,*4*,*7*,*8*). However, for serious and deep-seated *S. aureus* infections (e.g., bacteremia, endocarditis), vancomycin is inferior to β-lactam antimicrobial drugs, even when in vitro testing indicates susceptibility. Treatment failures have been encountered (*4*). Linezolid is a good alternative but limited by availability and cost, and clindamycin therapy can be associated with inducible resistance. For BORSA-associated infections, β-lactam antimicrobial drugs, including high-dose penicillinase-resistant penicillins (PRPs) (e.g., cloxacillin) or β-lactam/β-lactamase–inhibitor combinations (e.g., ampicillin/sulbactam) are regarded as treatments of choice (*4*,*6*,*9*).

BORSA initially described non-heteroresistant strains of *S. aureus* with oxacillin MIC ≤2 mg/L, which produce ample β-lactamases and are rendered fully susceptible to PRP by β-lactamase-inhibitors (*4*,*6*). Subsequent BORSA strains described have had higher oxacillin MICs (4–8 mg/L) (*4*). The proportion of BORSA among clinical isolates of *S. aureus* varies (1.4%–12.5%) but is usually ?5% (*4*,*10*). A BORSA infection outbreak among dermatology patients with severe skin diseases has also been reported (*10*). Postulated resistance mechanisms include overproduction of conventional penicillinases, production of an inducible, plasmid-mediated, membrane-bound methicillinase, and in some cases, point mutations of penicillin-binding-proteins (*4*). The clinical importance of BORSA is unknown since early clinical/animal data suggest treatment efficacy of PRP (against strains with MIC ≤2 mg/L) (*4*,*6*,*9*). Whether BORSA with higher oxacillin MICs (4–8 mg/L) will respond equally well to PRP is less clear. Further studies into the treatment of BORSA, including pharmacokinetic considerations, are needed (*4*). However, high-dose β-lactam/β-lactamase inhibitor combinations (e.g., ampicillin/sulbactam), as shown in animal models, are at least as effective as PRP (*9*). In conclusion, our report suggests that *mec*A (or PBP2a) detection may help manage serious, community-acquired, non–multidrug-resistant MRSA infections because of the potential confusion between BORSA and CA-MRSA.

Lee Nelson,* Clive S. Cockram,* Grace Lui,* Rebecca Lam,* Edman Lam,* Raymond Lai,* and Margaret Ip*

*Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong SAR, People's Republic of China

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Address for correspondence: Margaret Ip, Department of Microbiology, Prince of Wales Hospital, Chinese University of Hong Kong, Ngan Shing St, Hong Kong SAR, People's Republic of China; fax: 852-2647-3227; email: margaretip@cuhk.edu.hk

Rickettsia massiliae Human Isolation

To the Editor: The number of new rickettsial species that cause diseases in humans is rapidly increasing (*1*). Moreover, many of the species first described in ticks have been recently shown to be pathogenic. Of the 10 species or subspecies found to be pathogens after 1984, a total of 7 were first isolated from ticks (*2*). We report the first isolation of *Rickettsia massiliae* from a patient. The bacterium was isolated in Sicily in 1985 and identified in 2005.

A 45-year-old man was hospitalized in Palermo, Italy, on June 6, 1985, for fever and a rash. He had been febrile since May 25 and did not respond to antimicrobial drug treatment using cefamezin, a first-generation cephalosporin. On examination, he had a necrotic eschar on his right ankle, a maculopapular rash on his palms and soles (online Appendix Figure 1, available at http://www.cdc.gov/ncidod/EID/vol12no01/05-0850-G1.htm), and slight hepatomegaly. Leukocyte count was normal; he received tetracyclines for 13 days and fully recovered. He seroconverted (from 0 to 1:80 between day 11 and day 24) by indirect immunofluorescence to *Rickettsia conorii* (*R. conorii* spot, bioMérieux, Marcy l'Étoile, France).

Four milliliters of heparinized blood sampled before treatment were inoculated in a 25-cm^2 flask containing Vero cells and incubated at 33°C in a CO_2 incubator (*1*). Direct immunofluorescence test on a sample of the patient's serum was positive 7 days later. The strain was stored for 20 years and tested in 2005 at the Unité des Rickettsies for identification, and *R. massiliae* was identified. DNA was extracted from the cell culture supernatant and used as template in 2 previously described polymerase

chain reaction (PCR) assays that targeted a portion of the rickettsial *ompA* gene as well as a portion of the rickettsial *gltA* gene (*3*,*4*). Amplification products of the expected size were obtained from this extract but from no concurrently processed control materials, including 3 negative controls. DNA sequencing of the positive PCR products gave 100% identity with *R. massiliae* for *ompA* (GenBank accession no. RBU43792) and 99.9% homology for *gltA* (GenBank accession no. RSU 59720).

R. massiliae was first isolated from *Rhipicephalus* ticks in Marseilles (*5*). It is transmitted transovarially in *Rhipicephalus turanicus* (*2*). *R. massiliae* is commonly found in *Rhipicephalus sanguineus* or *R. turanicus* in France, Greece, Spain (identified as Bar 29) (*6*), Portugal, Switzerland, Sicily (D. Raoult, unpub. data), Central Africa, and Mali (*2*). *R. massiliae* may be commonly associated with these ticks, which are distributed worldwide.

R. massiliae is grouped phylogenically with *Rickettsia rhipicephali* and *Rickettsia aeschlimannii* (online Appendix Figure 2, available at http://www.cdc.gov/ncidod/EID/vol12no01/05-0850-G2.htm). Bacteria from this group have a natural resistance to rifampin that is associated with an *rpo*B sequence that is different from that of other rickettsiae. This isolate was not tested for antimicrobial drug susceptibly (*7*). Rifampin resistance leads us to believe that this isolate may cause a Mediterranean spotted fever–like disease that was described in children in Spain (*7*,*8*). Serologic findings were recently reported that showed some patients in Barcelona, Spain, with reactions that indicate *R. massiliae* (B29 strain) rather than *R. conorii* (*6*). However, serologic reactions are only presumptive; isolation from a patient is the required to initially describe a new disease (*9*).

This Sicilian index case shows that *R. massiliae* is a human pathogen. It contraindicates using rifampin to treat Mediterranean spotted fever in areas where *R. massiliae* is endemic, as it cannot as yet be differentiated from *R. conorii* infection. *R. massiliae* is a new example of a strain identified in ticks for several years before its first isolation from a human patient (*10*). The longest delay was observed for *Rickettsia parkeri*, which was isolated from ticks in 1939 but not from a patient until 2004. Many authors labeled *R. parkeri* a nonpathogenic rickettsia during this time (*1*). In the present case, the human isolate was obtained before the tick isolate but was not further identified. When this strain was isolated, *R. conorii* was the sole *Rickettsia* sp. found in ticks in southern Europe. Moreover, only 1 tickborne pathogenic *Rickettsia* sp. was believed to circulate in a single area. Since that time, several tickborne rickettsial diseases have been shown to exist in the same area, which prompted us to retrospectively identify this strain. The patient was reexamined in May 2005, after this identification. He is healthy and has no remaining antibodies against *Rickettsia* spp.

Giustina Vitale,*
Serafino Mansueto,*
Jean-Marc Rolain,†
and Didier Raoult†

*Azienda Ospedaliera Universitaria Policlinico "P. Giaccone," Palermo, Italy; and †Université de la Méditerranée, Marseille, France

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Address for correspondence: Didier Raoult, Unité des Rickettsies, CNRS UMR 6020, Faculté de Médecine, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille CEDEX 05, France; fax: 33-4-91-38-77-72; email: didier.raoult@medecine.univ-mrs.fr

Bertiella studeri Infection, China

To the Editor: *Bertiella* is a genus of tapeworm in the family *Anoplocephalidae*, many species of which exist as parasites of nonhuman primates. Two species of the genus, *Bertiella studeri* and *B. mucronata*, can infect humans (*1*). More than 50 cases of human infection have been recorded, and the geographic distribution of cases shows that the tapeworm exists in countries in Asia, Africa, and the Americas. We report a *B. studeri* infection in a person; to our knowledge, this case of bertiellosis is the first in China.

The patient was a 3.5-year-old Chinese boy from Suzhou City, Anhui Province. The boy had a 6-month history of frequent abdominal pain. His parents had noticed living "parasites" in his feces for 3 months; a segment of the worm was expelled every 2 or 3 days. According to the symptoms, doctors at the local hospital diagnosed his condition as *Taenia solium* infection and prescribed praziquantel, but no drug was available in the hospital or local drugstores. Consequently, the parents brought the child to Bengbu Medical College for further diagnosis and treatment.

The patient appeared healthy; routine medical examination showed normal heart, lung, liver, and spleen, and he had no fever. Though the patient had intermittent epigastric pain, the abdomen was soft and tender. A total of 133 proglottids were collected from the feces. Their average length was 0.1 cm, and the total length of all proglottids was 13 cm; each segment was 0.68–1.10 cm in width. Eggs (N = 53) were examined microscopically; they were roundish or oval, an average of 45.31 μm diameter (range 37.93–50.00 μm), and clearly showed typical pyriform apparatus, with visible hooklets (Figure). Other laboratory examinations showed hemoglobin level of 110 g/L, erythrocytes 3.9×10^{12} cells/L, and leukocytes 8.0×10^{9} cells/L. Although 2 species can parasitize humans, the geographic distribution and egg size of these species differ (*2*). *B. mucronata* has smaller eggs and is found only in the New World. On the basis of the size of the proglottids (*3*), larger eggs with pyriform apparatus and hooklets, and geographic distribution, the infecting cestode was identified as *B. studeri*.

The origin of infection was not confirmed; the only clue was that the boy's parents had once raised tame monkeys in a zoo. When the boy was 2 years old, he often played in the wildland, which is part of the zoo near the forest, and frequently fed and played with the captured monkeys. Further questioning showed that the boy had also been in frequent contact with wild monkeys. We could not confirm whether he had been infected by eating monkey food contaminated with mites.

The lifecycle of the cestode requires 2 hosts; nonhuman primates are generally the final host, while oribatid mites are the intermediate host, in which the infective cysticercoid of the cestode develops. Orbatid mites may exist in soil to maintain natural infection, and the definitive host is infected by eating or otherwise coming into contact with contaminated soil or food. Animal infection has been recorded in some provinces in China, and human bertiellosis has been recorded in Sri Lanka (*4*), Saudi Arabia (*5*), Vietnam (*6*), Japan (*7*), India (*8*), Thailand, Malaysia, and other Asian countries. However, according to the most recent Chinese authoritative text, Human Parasitology (*9*), no human bertiellosis has been recorded in China. Humans are infected by unconsciously swallowing infected mites, and in Mauritius, children were infected by eating guavas that had fallen on the soil (*10*). Other human infections may have occurred, but infected persons may have had mild symptoms and not noticed expelling the segments, so local doctors may have considered the cases to have been caused by a common cestode. To prevent human bertiellosis, the relationship between human cases and the natural host must be investigated.

Figure. Eggs collected from proglottids. Left panel shows the length of the egg, scale bar = 10μm; middle panel shows the hooklets in the egg; right panel shows the pyriform apparatus in the egg (under convert microscope).

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Xin Sun,*† Qiang Fang,*† Xing-Zhi Chen,*† Shou-Feng Hu,*† Hui Xia,*† and Xue-Mei Wang*†

*Bengbu Medical College, Bengbu, China; and †Anhui Provincial Key Laboratory of Infection and Immunity, Bengbu, China

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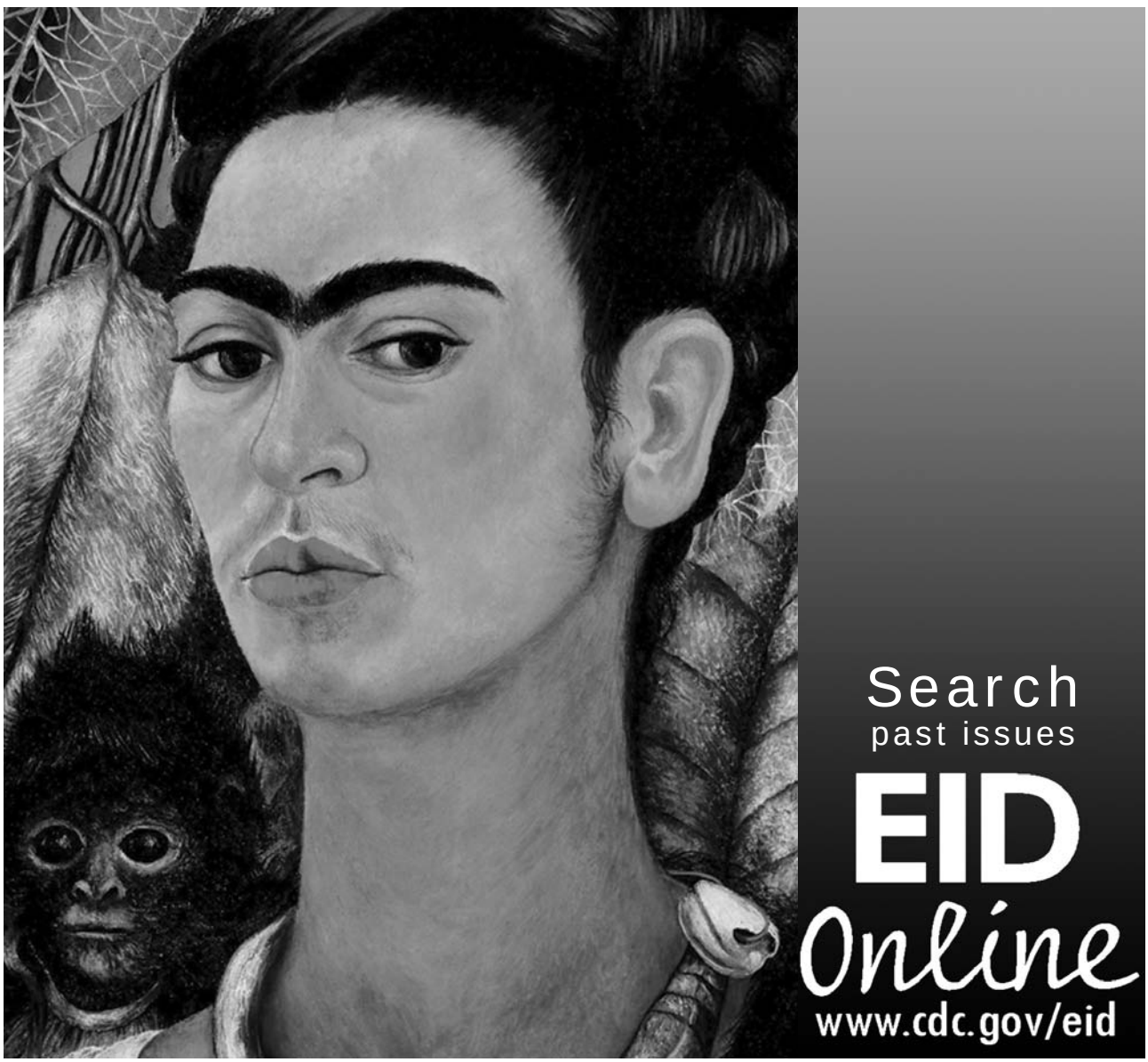
Address for correspondence: Xin Sun, Department of Parasitology, Bengbu Medical College, Anhui Provincial Key Laboratory of Infection and Immunity, 801 Zhihuai Rd, Bengbu 233003, China; fax: 86-552-317-1333; email: bengbusx@yahoo.com.cn



Molecular Pathogenesis of Virus Infections

Paul Digard, Anthony A. Nash, and Richard E. Randall, editors

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Molecular Pathogenesis of Virus Infections describes our current understanding of the pathogenesis of selected virus and prion infections. The innate response is an early barrier to virus spread. In this context, O. Haller et al. describe the antiviral activity of type I interferons and the various virus-encoded countermeasures. R.P. van Rij and R. Andino review the role of RNAi as a therapeutic antiviral agent and its use by the host and virus during viral infections. J.L. Whitton gives an overview of the adaptive CD8+ T-cell immune response in the context of virus infections. G. Screaton and J. Mongkolsapaya explain potential roles of T-cell responses in dengue hemorrhagic fever. E. Turnbull and P. Borrow provide a detailed description of the ineffectual roles of the innate and immune responses in the control of HIV and the long road ahead for development of either a prophylactic or therapeutic vaccine.

Transmissible spongiform encephalopathies have perhaps the most unconventional natural history of any infectious agent. J.C. Manson and R.M. Barron describe the diagnosis of transmissible spongiform encephalopathies, the appearance of new strains, and the nature of host susceptibility to disease. C.M. Dixon et al. depict the special problems presented to the host by certain RNA viruses that are maintained and persist in human populations through avoidance or inhibition of apoptosis, innate immune response, and adaptive immune response.

Other viruses infect humans only as incidental hosts and cause epizootics of varying degrees. A.L. Hartman et al. review our current understanding of the pathogenesis of Ebola and Marburg filoviruses, paying particular attention to the factors that contribute to lethal disease. C. Dye and S. Siddell discuss the pathogenesis of feline coronavirus, an animal disease model that has provided insights into the study of the newly recognized disease, severe acute respiratory syndrome. R.G. Webster et al. enumerate the key influenza genes responsible for human pathogenicity, their roles in past pandemics, and the potential of avian influenza virus strains to evolve into highly pathogenic and transmissible viruses for human populations.

Many viruses modify host metabolism and innate/immune responses to their own ends. L. Gray et al. describe the impact of human papillomaviruses on cell cycle and apoptosis. S.M. Lemon and K. Li review the data documenting hepatitis C virus disruption of innate intracellular antiviral defenses, including interferons and toll-like receptors. M.B. Ruiz-Arguello et al. enumerate the multiple, distinct receptor homologs and binding proteins encoded by poxviruses that target tumor necrosis factor. L.K. Dixon summarizes the multiple host pathways that are targeted at multiple levels by African swine fever virus. J.P. Stewart et al. describe the pathogenesis of murid herpesvirus 4 that supports its use as model for gammaherpesviruses. M.L. Freeman et al. provide an overview of the potential role of the immune system in the latency of the alphaherpesvirus, herpes simplex virus 1. This book is suitable for the serious student and professional and is well referenced for further reading.

Robert Buller*
*St. Louis University, St. Louis, Missouri, USA

Address for correspondence: Robert Buller, Department of Molecular Microbiology and Immunology, St. Louis University, M432, 1402 South Grand Blvd, St. Louis, MO 63104, USA: fax: 314-977-8717; email: bullerrm@slu.edu

The Germ Freak's Guide to Outwitting Colds and Flu: Guerilla Tactics to Keep Yourself Healthy at Home, at Work, and in the World

Allison Janse with Charles Gerba

Health Communications Inc, Deerfield Beach, FL, 2005
ISBN: 0-7573-0327-7
Pages: 194, Price: US $9.56

According to self-described "germ freak" Allison Janse, it's a dangerous world out there and I am not talking about Al Qaeda, anthrax in the mailbox, or Hurricane Katrina. The real danger, according to Janse, comes from elevator buttons (severe acute respiratory syndrome), escalator handrails (group B *Streptococcus*), subway platforms (*Aspergillus*) kitchen sinks (salmonella), loofah sponges (*Staphylococcus aureus*), and children's ball pits (*Escherichia coli*). She has a point, of course; everyday objects can transmit disease, but the value of her point is frequently lost in a hodgepodge approach that makes no distinction between serious but rare events, everyday avoidable ills, and the merely yucky.

In this book, Janse and her collaborator Charles Gerba use (sometimes badly misplaced) humor to alert us to the risks we run from everyday items like our kitchen cutting board ("If you have a choice between licking a cutting board or a toilet seat... pick the toilet seat" p. 50). The book does a nice job of addressing the overuse of antimicrobial drugs; encourages even blatant germ freaks to save their money and not buy antimicrobial soap for everyday use; is loaded with useful tips for reducing your family's vulnerability to sharing bugs of all sorts; provides a quick overview of the transmission, symptoms, and incubation period of some of the most common bugs (influenza virus, norovirus, cold viruses, and *E. coli*); and can induce a mania for handwashing among even the most hygiene-challenged. Unfortunately, the authors spend too much time on items that have no bearing on the transmission of colds and influenza, or anything else for that matter, and not nearly enough time providing detailed, "how-to" instructions.

Even less helpful is an entirely too flippant attitude toward the potentially valuable role that germ freaks can play in public health education. In a section entitled "Operation Germ Evasion," the authors provide a list of suggested responses that germ freaks should memorize, so they won't be caught off guard at a party when faced with ignorant comments from non–germ freaks. Two examples will suffice: 1) Non–germ freak comment, "I read about this hygiene hypothesis that says being too clean is causing increased illness." Suggested response, "I didn't think you knew how to read" (p. 29). 2) Non–germ freak comment, "Children who grow up in homes that are too clean are more likely to have asthma." Suggested response, "Then your kids are safe because your house is a real dump" (p. 29). These responses would not only give Miss Manners serious pause, they overlook a valuable opportunity to teach skills in addressing, and placing in proper context, the kernels of truth embedded in comments such as these. Rather than belittling non–germ freaks, the authors would have been better advised to give space to a balanced discussion.

Ultimately, the best audiences for this book are fellow germ freaks, who will enjoy the social validation it provides, and persons with enough preexisting savvy about infectious disease to sort out the helpful tips from the overly dramatic prose ("When you touch the shopping cart handle laden with *E. coli* and then sample the deli turkey, your life could literally be in your own hands" [p. 15]). In context, this book is entertaining and informative, but I would not recommend it for general consumption, without prior sanitization by the informed.

Kimberly Sessions*

*Emory University, Atlanta, Georgia, USA

Address for correspondence: Kimberly Sessions, Rollins School of Public Health of Emory University, 1518 Clifton Rd NE, Atlanta, GA 30329, USA; fax: 404-727-9853; email: kbs@emory.edu

etymologia

influenza

[in″floo-en′zə]

Acute viral infection of the respiratory tract. From Latin *influentia*, "to flow into"; in medieval times, intangible fluid given off by stars was believed to affect humans. The Italian *influenza* referred to any disease outbreak thought to be influenced by stars. In 1743, what Italians called an *influenza di catarro* ("epidemic of catarrh") spread across Europe, and the disease came to be known in English as simply "influenza."

Sources: Dorland's illustrated medical dictionary. 30th ed. Philadelphia: Saunders; 2003 and Quinion M. World wide words. 1998 Jan 3 [cited 2005 Dec 5]. Available from http://www.worldwidewords.org/topicalwords/tw-inf1.htm

Winslow Homer (1836–1910). Right and Left (1909) (detail). Oil on canvas (.718 m × 1.229 m).
National Gallery of Art, Washington, DC. Gift of the Avalon Foundation

Painting Nature on the Wing

Polyxeni Potter*

"The Sun will not rise, or set, without my notice, and thanks," wrote Winslow Homer to his brother Charles, putting into words what drove his art (*1*). All his life, the artist "noticed"—not just the rising and setting sun from his home near the ocean at Prouts Neck, Maine, but everything around him: people; scenery of his hunting and fishing trips; wildlife, which he painted with passion; objects and details others would never imagine the subject of art; and the effect, movement, and inner life of these. As he gave drawing instructions to his friend and fellow artist James Edward Kelly (1855–1933), Homer once said, "You should practice drawing old shoes and getting their character….You should practice drawing high hats…. There is a great deal of drawing in a high hat, to get not only its curves, but its delicate variations in the outline which gives it style" (*2*).

In Cambridge, Massachusetts, where he grew up, Homer was exposed to art and encouraged to draw at an early age. His mother, Henrietta Benson, a successful illustrator of flowers and other still life, taught him watercolor painting and foresaw his "future greatness." Hers were the only works, besides his own, found in Homer's studio after his death. As a youth, he was apprenticed to a Boston lithographer and later took evening classes at the National Academy of Design in New York, but he was primarily self-taught, his career marked by continued growth (*3*).

At age 21, Homer became a freelance illustrator and in no time he was working for the prestigious Harper's Weekly. Soon, the Civil War broke out. Sent to the battlefields as artist-correspondent, he turned his observant eye not to war action and combat but to soldiers' plight. He sketched from life at camp then converted sketches into engravings at his studio in New York. Documenting drab labor behind the lines and the soldiers' loneliness and alienation gained him national recognition.

"Quite late this man went to Europe and studied there and found things ready to his hand, but I do not know what more he got beyond what he had already," wrote John LaFarge (1835–1910), Homer's friend and fellow artist (*2*). Homer's time in Paris, after the war, and in Cullercoats, an English fishing village and artists' colony on the North Sea, had little influence on his style. "A great man," LaFarge believed, "…has left for us what I think is the only record of absolutely American Yankee expression" (*2*).

"You will see, in the future I will live by my watercolors," Homer remarked when, already accomplished in the weightier world of oils, he returned to the more direct medium of his childhood (*4*). The freshness and lightness

*Centers for Disease Control and Prevention, Atlanta, Georgia USA

of watercolor lent itself more naturally to his vision. He painted common people and bucolic scenes, the innocence of an era quickly disappearing from 19th-century America. "Barbarously simple," said Henry James. "He has chosen the least pictorial features of the least pictorial range of scenery and civilization as if they were every inch as good as Capri or Tangier; and, to reward his audacity, he has incontestably succeeded" (*4*).

Homer's interest in watercolor painting matured at his Prouts Neck studio, a refurbished stable off the family estate. In addition to "wearing out the deck" of his home pacing as he observed ocean storms, he traveled to Nassau, Cuba, Florida, Bermuda, interpreting the bright Caribbean light on the exotic scenery, omitting human figures, showing them in their struggle against the forces of nature, or displaying their fragility against vast expanses of sea and sky. He became one of the best watercolorists in the world.

An avid sportsman and naturalist, Homer was also master of the wildlife genre, best at both observing and identifying with the sporting landscape. He traveled with palette and brushes, painting nature on the wing. From Prouts Neck to the Adirondacks, from Quebec to the Florida Keys, he captured bird and trout, the woods, the whitetail, the common duck, identifying with the hunters and the hunted, marveling at and fearing nature, outlining a structured landscape that was uniquely simple and grand. "Homer," his biographer Lloyd Goodrich wrote, "…employed the affirmative elements of the American spirit…. He did for our painting what Walt Whitman did for our poetry—he made it native to our own earth and water" (*5*).

The illusion suggested by the artist's work is directed by him but mostly made by us," wrote LaFarge, suggesting that looking at a work of art is not passive for it involves the viewer's imagination (*2*). And since viewer cannot be separated from art, preconception or lack of "knowing" can interfere with appreciation. In his small circle of friends, Homer was dubbed "the obtuse bard." An allusion to his Hellenic namesake, this description fit a certain poetic license in his work to say one thing and mean another, as well as his often misunderstood attempts at humor, which, like his paintings, allowed multiple interpretations.

Right and Left, on this month's cover, was painted a year before Homer's death (*6*). The title is hunt jargon for using a double-barreled gun to shoot two ducks in rapid succession. The hunter, on the waves in some distance, is barely visible behind the flare of the shotgun. We witness the aquatic scene from the birds' perspective in the sky. Bird on the right, possibly struck first, falls limply toward the ocean. Bird on the left, in direct range, makes desperate attempt at exit as the second shot is fired. Or is the bird on the left stunned from being hit first, in the back, while the other bird is diving to escape?

The "in your face" travel of the birds and bullets adds dramatic immediacy. Agitated waters, a glaring eye, the rocking boat underline violence. Dislodged feather and ray of sunshine mark the fleeting moment. This scene, painted when death must have been on Homer's mind, seems the culmination of a lifetime of observation.

"Good metaphor," noted Aristotle in his Poetics, "implies an intuitive perception of the similarity of dissimilars," it implies likeness (*7*). A bird is not human, but a single element in its appearance can invoke humanity, just as a single element in a plant's appearance can distinguish its species. Homer's masterful hunting scene, commissioned as a sporting picture, is much more.

In a few brushstrokes, the artist delivers the ocean's power, the vastness of creation, conflict in the world, riddles in nature. He projects the birds and their plight, the hunter's unimportance, even as he fires the fatal shots. The ambiguity in their posture is the artist's ambivalence about which bird died first. The threat is imminent and inevitable. Death is certain.

The artist's vision holds yet more ambiguity today. The sporting ducks deliver as well as receive havoc. When they escape the double-barreled shotgun and fly off, they may carry with them nature's revenge, introducing new flu virus strains right and left: to domestic animals or directly to humans, increasing risks for new pandemics. As we stare into the hunter's barrel in Homer's painting, we could be the sitting ducks.

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Address for correspondence: Polyxeni Potter, EID Journal, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D61, Atlanta, GA 30333, USA; fax: 404-371-5449; email: PMP1@cdc.gov

EMERGING INFECTIOUS DISEASES

Upcoming Issue

Look in the February issue for the following topics:

Antimicrobial Drug Resistance, Regulation, and Research

HIV Drug-resistant Strains as Epidemiologic Sentinels

Detecting Hepatitis B Surface Antigen Mutants

Systematic Review of Antimicrobial Drug Prescribing in Hospitals

Bartonella quintana Characteristics and Clinical Management

Rickettsia africae in the West Indies

Free-grazing Ducks and Highly Pathogenic Avian Influenza

Nipah Virus and Encephalitis Outbreak, Siliguri, India

Methicillin-resistant *Staphylococcus aureus* Clones, Western Australia

Amoeba-associated Microorganisms and Diagnosis of Nosocomial Pneumonia

Rickettsial Infections and Fever, Vientiane, Laos

Ophthalmic Complications of Dengue

Helicobacter pylori in Chickens, Belgium

Epizootiologic Parameters for Plague in Kazakhstan

Pediatric Blastomycosis, Ontario

Complete list of articles in the February issue at http://www.cdc.gov/ncidod/eid/upcoming.htm

Upcoming Infectious Disease Activities

February 5–9, 2006
13th Conference on Retroviruses and Opportunistic Infections
Colorado Convention Center
Denver, CO, USA
http://www.retroconference.org

March 16–18, 2006
International Conference on Women and Infectious Diseases: Progress in Science and Action
Marriott Marquis Hotel
Atlanta, GA, USA
http://www.womenshealthconf.org

March 19–22, 2006
International Conference on Emerging Infectious Diseases 2006
Marriott Marquis Hotel
Atlanta, GA, USA
http://www.iceid.org

March 22–24, 2006
International Symposium on Emerging Zoonoses
Medical and Veterinary Partnerships To Address Global Challenges
Marriott Marquis Hotel
Atlanta, GA, USA
http://www.isezconference.org

May 19–23, 2006
Council of Science Editors 49th Annual Meeting
Hyatt Regency New Orleans
New Orleans, LA, USA
http://www.councilscienceeditors.org

June 17–22, 2006
Negative Strand Viruses 2006: Thirteenth International Conference on Negative Strand Viruses
Salamanca, Spain
Contact: 404-728-0564 or meeting@nsv2006.org
http://www.nsv2006.org

June 25–29, 2006
ISHAM 2006 (International Society for Human and Animal Mycology)
Palais des Congrès
Paris, France
Contact: 770-751-7332 or c.chase@imedex.com
http://www.imedex.com/calendars/infectiousdisease.htm

EMERGING INFECTIOUS DISEASES

www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

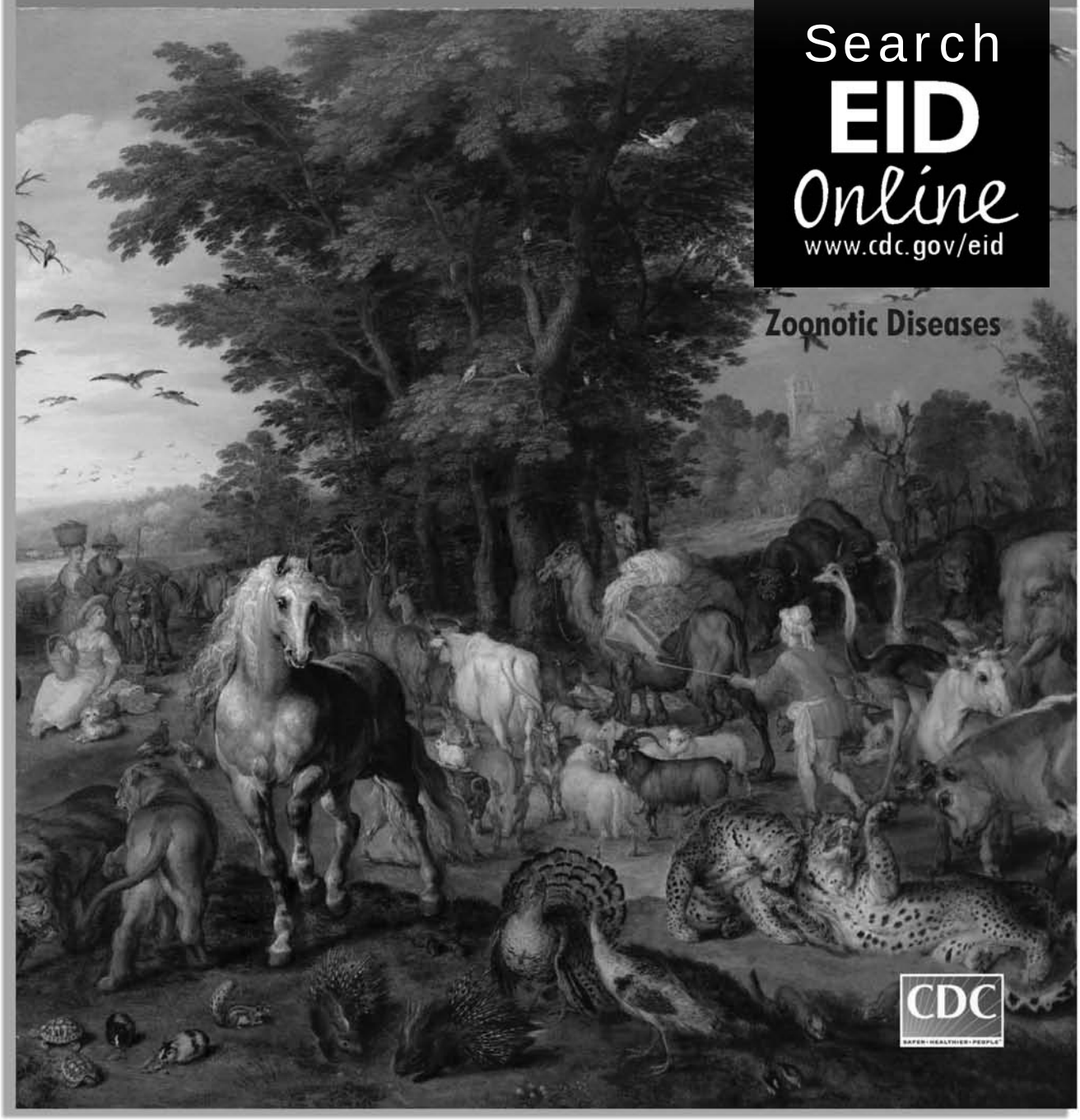
   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

EMERGING INFECTIOUS DISEASES

EID Online www.cdc.gov/eid

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Vol.11, No.12, December 2005



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Zoonotic Diseases

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EMERGING INFECTIOUS DISEASES
A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Editorial Policy and Call for Articles

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles see below and visit http://www.cdc.gov/eid/ncidod/ EID/instruct.htm.

Emerging Infectious Diseases is published in English. To expedite publication, we post articles online ahead of print. Partial translations of the journal are available in Japanese (print only), Chinese, French, and Spanish (http://www.cdc.gov/eid/ncidod/EID/trans.htm).

Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. Begin each of the following sections on a new page and in this order: title page, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje. org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by “et al.” Do not cite references in the abstract.

Tables and Figures. Create tables within MS Word’s table tool. Do not format tables as columns or tabs. Send graphics in native, high-resolution (200 dpi minimum) .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) format. Graphics should be in a separate electronic file from the text file. For graphic files, use Arial font. Convert Macintosh files into the suggested PC format. Figures, symbols, letters, and numbers should be large enough to remain legible when reduced. Place figure keys within the figure. For more information see EID Style Guide (http://www.cdc.gov/ncidod/ EID/style_guide.htm).

Manuscript Submission. Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch) and verifying that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid).

Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch of first author—both authors if only 2. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief biographical sketch of first author—both authors if only 2. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., “The Study” and “Conclusions.” Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and a brief biographical sketch of first author—both authors if only 2. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Book Reviews. Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted on the journal Web page only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.