Are We Ready for Pandemic Influenza?

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During the past year, the public has become keenly aware of the threat of emerging infectious diseases with the global spread of severe acute respiratory syndrome (SARS), the continuing threat of bioterrorism, the proliferation of West Nile virus, and the discovery of human cases of monkeypox in the United States. At the same time, an old foe has again raised its head, reminding us that our worst nightmare may not be a new one. In 2003, highly pathogenic strains of avian influenza virus, including the H5N1 and H7N7 subtypes, again crossed from birds to humans and caused fatal disease. Direct avian-to-human influenza transmission was unknown before 1997. Have we responded to these threats by better preparing for emerging disease agents, or are we continuing to act only as crises arise? Here we consider progress to date in preparedness for an influenza pandemic and review what remains to be done. We conclude by prioritizing the remaining needs and exploring the reasons for our current lack of preparedness for an influenza pandemic.

In February 2003, during a family visit to mainland China, a young girl from Hong Kong died of an unidentified respiratory illness. After returning to Hong Kong, both her father and brother were hospitalized with severe respiratory disease, which proved fatal to the father. When H5N1 (avian) influenza virus was isolated from both patients, the World Health Organization (WHO) went to pandemic alert status (*1*). At about the same time, there were rumors of rampant influenza-like disease in China. Influenza experts feared that H5N1 influenza virus had acquired the ominous capacity to pass from human to human. That outbreak is now known to have been SARS, caused by a novel coronavirus.

In March 2003, another alarming situation arose on the other side of the world. A highly pathogenic H7N7 avian influenza outbreak had recently erupted in the poultry industry of the Netherlands (*2*), and workers involved in the slaughter of infected flocks contracted viral conjunctivitis. The H7N7 virus isolated from these patients had several disquieting features: Not only could it replicate in the human conjunctiva, but there was also evidence of human-to-human spread. Nearby herds of swine (which are often implicated in the adaptation of influenza viruses to humans) also showed serologic evidence of exposure (*2*). When a veterinarian died of respiratory infection (*2*–*5*), WHO again acknowledged the presence of a severe threat (*6*).

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Luckily, the worst-case scenarios did not come about in either of the 2003 avian influenza virus scares. However, the year's events eliminated any remaining doubts that global advance planning for pandemic influenza is necessary. They also highlighted how far, as a scientific community, we have come since the 1997 event: We are now much better equipped with technologies and reagents to rapidly identify and respond to pandemic influenza threats. On the other hand, the legislative and infrastructure changes needed to translate these advances into real public health benefits are alarmingly slow.

The Role of WHO in Influenza Surveillance and Control

In 2001, WHO initiated the development of a Global Agenda for Influenza Surveillance and Control. Its four main objectives are to strengthen influenza surveillance, improve knowledge of the disease burden, increase vaccine use, and accelerate pandemic preparedness (*7*). In May 2002, this document was adopted after proposals and public comment were invited. The document advocates the development of methods and reagents that can be used to rapidly identify all influenza virus subtypes, thereby allowing integrated influenza surveillance in humans and in other animals. WHO, with its global influenza network of more than 100 laboratories and its distinguished record of planning for yearly interpandemic influenza, is ideally situated to play a broader role in facilitating international cooperation for the rapid exchange of viruses, reagents, and information. Influenza continually evolves at the human–lower animal interface and thus can be unpredictable. As an example, within a brief period, the H7N7 virus events occurred in European poultry and humans, H5N1 viruses infected Asian poultry and humans, and novel, rapidly spreading reassortant viruses were isolated in swine in the United States (*8*, *9*). Therefore, the capacity to simultaneously manage multiple potential pandemic situations is important. The WHO global agenda document will help to prioritize areas of influenza research and facilitate national pandemic preparedness plans.

Prioritization of Viral Subtypes for Surveillance and Control

Influenza experts agree that another influenza pandemic is inevitable and may be imminent (Fig. 1). A major challenge in controlling influenza is the sheer magnitude of the animal reservoirs. It is not logistically possible to prepare reagents and vaccines against all strains of influenza encountered in animal reservoirs, and therefore, virus subtypes must be prioritized for pandemic vaccine and reagent preparation. Preliminary findings have identified the H2, H5, H6, H7, and H9 subtypes of influenza A as those most likely to be transmitted to humans. [Influenza viruses are typed according to their hemagglutinin (H) and neuraminidase (N) surface glycoproteins.] The influenza A subtypes currently circulating in humans, H1 and H3, continue to experience antigenic drift. That is, their antigenic surface glycoproteins are continually modified, allowing them to escape the population's immunity to the previous strain and thus to continue causing annual outbreaks. Although these continual modifications may lead to an increase in virulence, the mildness of the past three influenza seasons suggests that the dominance of the H1N1 and H3N2 viruses is waning as their ability to cause serious disease becomes increasingly attenuated. H2 influenza viruses are included in the high-risk category because they were the causative agent of the 1957 "Asian flu" pandemic and were the only influenza A subtype circulating in humans between 1957 and 1968. Counterparts of the 1957 H2N2 pandemic virus continue to circulate in wild and domestic duck reservoirs. Under the right conditions (which are still not completely understood), H2N2 viruses could again be transmitted to and spread among humans, none of whom under the age of 30 years now has immunity to this virus. Seroarchaeology data from the late 19th and early 20th centuries indicate that only the H1, H2, and H3

influenza virus subtypes have been successfully transmitted among humans. It is possible, but unlikely, that they are the only subtypes able to do so.

Not only are the H1, H2, and H3 influenza viruses of concern, but the H5 subtype has threatened to emerge as a human pandemic pathogen since 1997, when it killed 6 of 18 infected humans. Before that event, the receptor specificity of avian influenza viruses was thought to prevent their direct transmission to humans. Transmission from aquatic birds to humans was hypothesized to require infection of an intermediate host, such as the pig, that has both human-specific (α2-6 sialic acid) and avian-specific (α2-3 sialic acid) receptors on its respiratory epithelium. The 1997 H5N1 event demonstrated that domestic poultry species may also act as intermediate hosts. H5N1 viruses continue to emerge and evolve despite heroic measures taken to break their evolutionary cycle in the live poultry markets of Hong Kong: the elimination of live ducks and geese (the original source), the elimination of quail (the source of the internal genes of H5N1/97), and the institution of monthly "clean days," when all 1000-plus retail markets are emptied and cleaned.

Two things have become clear. Live poultry markets are potential breeding grounds for influenza and other emerging disease agents, and there is an Asian source of H5N1 influenza viruses outside of Hong Kong SAR. Between 1997 and 2003, H5N1 virus was isolated from duck meat imported from China into Korea (*10*) and Japan (*11*). These observations suggest that ducks and possibly other avian species in mainland China are a reservoir of H5N1, although there have been no official reports of H5N1 virus in China.

At the beginning of the SARS outbreak, China missed an opportunity to show the world its considerable intellectual and scientific potential (*12*). In the case of H5N1 influenza, a pandemic in waiting, it remains to be seen whether China will show leadership in proactively addressing the problem. Concerted national and international efforts are required to deal effectively with the threat.

The third virus subtype on the most wanted list is H7. The H7 and H5 viruses have a unique ability to evolve into a form highly virulent to chickens and turkeys by acquiring additional amino acids at the hemagglutinin (HA) cleavage site (HA cleavage is required for viral infectivity) (*13*). The highly pathogenic H7N7 influenza viruses that were lethal to poultry infected the eyes of more than 80 humans and killed one person (*14*). In the case of this outbreak, the Netherlands' policy of openness was important in reducing the potential threat and should serve as a model. When the virus was first detected at the end of February 2003, the European Community and international community, via the Office International des Epizooties, were notified so that surrounding countries, including Belgium and Germany, could immediately respond if the disease was detected. Culling of all poultry on infected farms and quarantine of surrounding farms succeeded in eradicating the virus once the etiologic agent was identified. After human infection was observed, an anti-influenza drug was given as prophylaxis, and vaccination with the current human influenza vaccine was done to reduce the likelihood that the avian virus would reassort with human H1N1 and H3N2 strains.

The remaining two viral subtypes on the priority list, H6 and H9, do not share the virulent phenotypes of the H5 and H7 viruses, but still pose a considerable threat. Both of these influenza viruses have spread from a wild aquatic bird reservoir to domestic poultry over the past 10 years. H9N2 viruses have also been detected in humans and in pigs (*15*, *16*) and have acquired human-like receptor specificity (*17*). Neither of these viruses was able to infect chickens before the mid-1980s. Now, for unknown reasons, H9 viruses are endemic in chickens in Eurasia and H6 viruses are becoming endemic in both Eurasia and the Americas. These facts highlight the continuing adaptation of influenza viruses in the aquatic bird reservoirs to domestic chickens.

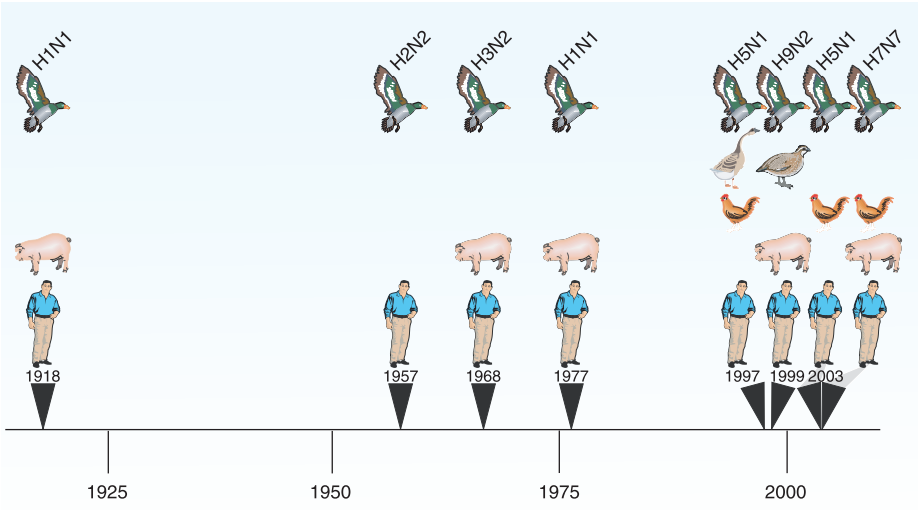

Fig. 1. Timeline of human influenza over the past 100 years. The black triangles represent documented human influenza A infections characterized by multiple cases. In each instance the species of animals implicated in the emergence of disease is highlighted. Since 1997 there has been a disproportionate increase in the number of reports of novel subtypes in humans and in the number of animal and bird species involved, suggesting that the next influenza pandemic is imminent.

The Challenge of Developing Candidate Vaccines

If the next influenza pandemic were to begin tomorrow, inactivated vaccines would offer the only immediate means of mass prophylaxis, yet their supply is limited by inadequate production capabilities and suboptimal utilization of adjuvants (*18*, *19*). The stocks of antiviral drugs are too low to cope with an epidemic and would be quickly depleted (*19*). Tissue culture–based and live attenuated vaccines are now licensed in some countries, and could supplement the supply of inactivated vaccine. Further development of these options is urgently needed to provide alternative substrates in the face of a pandemic.

Since the 1970s, influenza vaccines have been made by exploiting the tendency of the segmented influenza genome to reassort (*20*). This natural process has been used to produce vaccine strains that simultaneously contain gene segments that allow them to grow well in eggs and gene segments that produce the desired antigenicity. Natural reassortment is allowed to occur in embryonated chicken eggs, and reassortants with the desired characteristics are selected. These recombinant vaccine strains contain the hemagglutinin and neuraminidase genes of the target virus (encoding glycoproteins that induce neutralizing antibodies); their remaining six gene segments come from A/Puerto Rico/8/34 (H1N1), which replicates well in eggs and is safe for use in humans (*21*). These "6+2" reassortants are then grown in large quantities in embryonated chicken eggs, inactivated, disrupted into subunits, and formulated for use as vaccines. Although this process creates an effective and safe influenza vaccine, it is too time-consuming and too dependent on a steady supply of eggs to be reliable in the face of a pandemic emergency. Even during interpandemic periods, 6 months is required to organize sufficient fertile chicken eggs for

annual vaccine manufacture (*22*), and the preparation of the desired "6+2" recombinant vaccine strain can be a time-consuming process. Influenza vaccine preparation is seasonal and is a remarkable achievement, in that an essentially new vaccine is made every year. However, two of the viruses of greatest concern, those of the highly pathogenic H5 and H7 subtypes, cannot be successfully grown in eggs. Their unique ability to accumulate multiple basic amino acids at the site of hemagglutinin cleavage increases their ability to spread systemically in an infected host and cause significant disease (*13*). This feature also renders H5 and H7 viruses rapidly lethal to chicken embryos.

The most promising means of expediting the response to pandemic influenza is the use of plasmid-based reverse genetic systems to construct influenza virions and vaccines. These systems also offer a successful alternative means of producing H5 and H7 vaccine seed strains. Because viable viruses can be generated from individually cloned cDNA copies of each of the eight viral RNA segments, reassortment can be prospectively defined and directed, and the extra amino acids at the HA cleavage site (which are associated with high virulence) can be removed to allow rapid generation of a vaccine seed strain in eggs. Plasmids encoding the internal genes of the base vaccine are already available. A vaccine seed strain can be created by cloning the appropriate hemagglutinin and neuraminidase genes from the target virus, altering its HA connecting peptide if necessary, and transfecting an appropriate cell line (Fig. 2). This technology has been shown to be effective for the production of reassortants carrying several different surface glycoprotein combinations, including those considered to have a high pandemic potential (*23*–*26*). The next step is to take these plasmid-derived influenza vaccines through clinical trials to address crucial questions such as number and quantity of doses and the role of adjuvants. Most of the vaccines derived after the 1997 H5N1 episode by various alternative strategies induced a disappointing immune response (*27*). The optimal pandemic vaccination regimens can be anticipated only by collecting the necessary data and experience through clinical trials of vaccines against different subtypes of influenza virus.

Although they are well suited to the manufacture of inactivated influenza vaccines, reverse genetic systems introduce new variables. One of the most limiting of these is the need to use cell lines. There are surprisingly few suitable accredited cell lines and cell banks available, and many of those are the property of pharmaceutical companies. The practical options are very few, in view of the technical and regulatory restrictions. Perhaps the only cell line that meets all criteria for international use at this time is the African green monkey kidney cell line, Vero. However, although Vero cell lines are in widespread laboratory use, only those that are derived from WHO-approved sources and have a detailed history are acceptable for manufacture of human pharmaceuticals. A second new variable is the use of a genetically modified virus seed strain. Because the traditional vaccine strains are made by natural reassortment, they have escaped being labeled "genetically modified." This difference, although largely semantic, may affect the acceptance of the new vaccines. Before many of these traits can be tested, the virus must be amplified, inactivated, purified, and formulated for vaccine use (*22*).

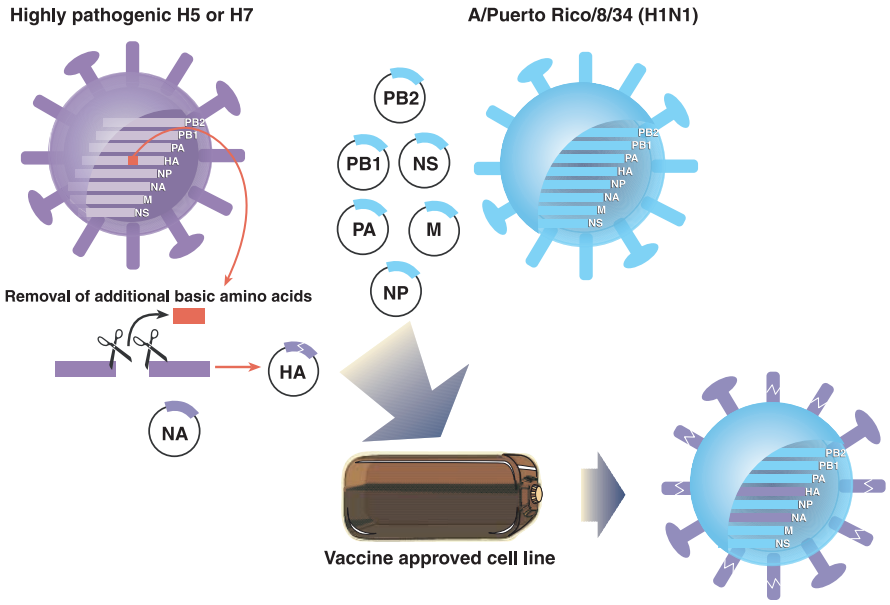

Fig. 2. Proposed method of influenza vaccine seed virus production using the eight-plasmid reverse genetics system (*23*). The hemagglutinin (HA) and neuraminidase (NA) genes from the target strain are cloned into the bacterial plasmid vector pHW2000 in a process that allows for the alteration of the HA cleavage site when necessary (see text for explanation). These two plasmids, along with six others containing the remaining influenza A gene segments derived from the master vaccine strain A/Puerto Rico/8/34 (H1N1), are then introduced into a suitable cell line (e.g., Vero). After expression of positive- and negative-sense RNA and viral proteins from these plasmids, a productive replication cycle is initiated and viable virus particles are produced.

In preparing for a pandemic threat, collaboration between government, industry, and academia is needed to overcome the obstacles and guarantee the most rapid production of a vaccine candidate. The recent SARS episode has shown that international collaboration in the face of a truly global threat is indeed possible.

The Safety Testing of Candidate Pandemic Vaccines and Liability Issues

Unfortunately, there are only a few facilities available to carry out safety testing under the high-level biocontainment conditions required for handling highly pathogenic influenza viruses. Overcoming the technical hurdles to efficient vaccine production is only the start of a long, expensive process. Manufacturing scale-up presents its own problems, not least because plant workers will have no immunity to the pathogens they will be handling. Of prime importance is vaccine safety testing, but the need for safety testing will have to be balanced against the need for rapid mass production of a vaccine. In response to the 2003 H5N1 scare in Hong Kong, WHO has created an Interim Biosafety Risk Assessment (*28*) guideline for the safety testing of pandemic vaccines, particularly the H5 and H7 subtypes, signifying a substantial advance in preparedness for the production of a pandemic influenza vaccine.

A major risk for all vaccine manufacturers is the occurrence of adverse reactions in a percentage of recipients. These reactions may be attributable to the vaccine, to the host, or (most likely) to a unique combination of the vaccine and the host genetic factors. Guillain-Barré syndrome in human beings first became apparent during the U.S. swine influenza vaccination program (*29*, *30*). The inevitability of adverse reactions underscores the product liability dilemma inherent in any vaccine program. The risk of devastating financial liability, and the unavailability or high cost of liability insurance, are increasingly discouraging vaccine manufacture, especially for universal use.

Legislative measures can be taken to reduce the impact of liability exposure. For example, the U.S. Congress passed the National Childhood Injury Compensation Act of

1986 (the "Vaccine Act"), which created a no-fault compensation program funded by an excise tax on vaccines. Plaintiffs need only establish that their injuries were caused by the vaccine. Claimants who are not satisfied with the administrative decision may still elect to sue the manufacturer, but the legal arguments available to the claimant are limited. Although the Vaccine Act represents progress in achieving a balance between consumer and manufacturer concerns, it would not apply to vaccines given to the general population, such as those for influenza or smallpox. Congress again attempted to address these concerns in a provision of the Homeland Security Act of 2002, and an Institute of Medicine panel is currently wrestling with the problem as well; however, drug manufacturers remain hesitant. The bottom line is that unless the government authorities of every country implement mechanisms that equitably limit vaccine liability, no prospective vaccine for H5N1, H7N7, or any other threatening influenza virus is likely to be produced for universal human use. It is hoped that governments will rise to the occasion after a crisis emerges, but logic suggests that the issue should be addressed now.

Antiviral Drugs

A global influenza strategy would call for the stockpiling of influenza antiviral drugs for use in the event of a pandemic until vaccines can be prepared. "But," as noted by Albert Osterhaus (*31*), "no country has yet started to stockpile antiviral drugs." The potential value of antivirals was demonstrated in the recent H7N7 outbreak in poultry and humans. Further, because epidemiological modeling has suggested that it is more infectious than SARS (*32*–*34*), influenza is unlikely to be controllable by SARS-like quarantine measures. The estimated 10 billion U.S. dollar cost of SARS and the societal disruption it caused in China and Toronto make a compelling case for stockpiling of antiviral drugs.

Pandemic influenza has already threatened twice in 2003. The events associated with these outbreaks show that we are in a much better position to rapidly respond to an influenza threat than we were in 1997; however, much remains to be accomplished. Overall, our state of preparedness is far from optimal.

Priorities to Ensure Pandemic Preparedness

To conclude, let us revisit our concern that the next influenza pandemic alert may involve a virus that has acquired the capacity to spread from human to human. What are our most urgent needs?

1) A sufficiently large supply of anti-influenza drugs to reduce the severity and spread of infection. Specific efficacious drugs are available, but no country has yet invested in stockpiling.

2) A vaccine matching the subtype of the emerging pandemic influenza strain that has been tested in clinical trials and for which manufacturers are prepared to "scale up" production. Such a vaccine would probably not match the emerging strain antigenically and would not prevent infection, but it could reduce the severity of illness until a matching vaccine is produced. Such vaccines have been discussed for 20 years. None is available, but specific plans to produce such a vaccine are currently being formulated.

3) The preparation, testing (safety and clinical trials), and availability of a vaccine derived by reverse genetics. The scientific technology is in place to achieve this goal, but manufacturing, intellectual property, and liability issues remain unresolved. In the event of a pandemic, reverse genetics would be the most rapid means by which to produce an antigenically matched vaccine. To be truly prepared, such a vaccine needs to be produced and tested now to identify and resolve the issues, rather than doing so in direct response to an emergency.

4) An improvement in the global influenza vaccine manufacturing capacity. Without the use of adjuvants, the current capacity is inadequate and could not be quickly augmented. The country best prepared to meet this need is Canada; in Ontario, influenza vaccination is recommended and available at no charge to people of all ages during the influenza season (*35*). This progressive strategy during interpandemic years will ensure the vaccine-manufacturing capacity of that region.

The conclusion of this analysis is inescapable: The world will be in deep trouble if the impending influenza pandemic strikes this week, this month, or even this year. It is now time to progress from talking about pandemic vaccines to taking action. Our hope is that the "Ontario experiment" will inspire other regions of the world to similarly promote the expansion of manufacturing capacity for influenza vaccines.

Although reverse genetics offers great advantages for the rapid preparation of influenza vaccine strains and for understanding pathogenesis (*36*), the reverse side of this benefit is its potential for the development of bioterrorism agents (*37*). Regardless of human endeavors, nature's ongoing experiments with H5N1 influenza in Asia and H7N7 in Europe may be the greatest bioterror threat of all. The time for talking is truly over. We must be prepared.

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