PREPARING FOR THE INFLUENZA PANDEMIC

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THE PANDEMIC THREAT

Influenza pandemics, which over time have occurred at irregular and unpredictable intervals, have been associated with substantial human morbidity, mortality, and social disruption, as well as with significant economic losses (see *1* for a review). In the 20th century, the world confronted three influenza pandemics: the 1918–1919 "Spanish flu" (A/H1N1) pandemic, the 1957 "Asian flu" (A/H2N2) pandemic, and the 1968 "Hong Kong flu" pandemic. The "Spanish flu" pandemic, by far the most devastating, caused acute illness in 25%–50% of the world's population and resulted in the death of more than 40 million people worldwide (roughly 1%–2% of the world's population); it brought an unusually high mortality among young adults. Mortality in the subsequent "Asian flu" and "Hong Kong flu" pandemics was considerably less—about 1–4 million people in each—and the highest excess mortality was among the classical risk groups, such as the elderly and people with chronic disease. Nevertheless, these two pandemics were associated with considerable morbidity, social disruption, and economic loss. Current understanding of the biology, ecology, and epidemiology of influenza A viruses indicates that we can assume that influenza pandemics will occur in the future, although at present it is impossible to predict when the next influenza pandemic will

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strike nor from which influenza A virus it will originate. Rapid changes in human behavior and animal ecology may even predispose the world for a more rapid spread of an influenza pandemic when it emerges. Recent advances in the development of effective antiviral drugs and vaccines using state of the art technology, as well as better surveillance in humans and animals, should provide us with more effective tools to combat a future influenza pandemic.

Wild birds are the reservoir for subtypes of influenza A viruses. To date, influenza A viruses carrying 16 antigenic subtypes of hemaglutinin (HA) and 9 antigenic subtypes of neuraminidase (NA) have been identified in wild aquatic birds and poultry *(2)*. Since 1997, epidemiologic investigations have pointed at the direct transmission of avian influenza A viruses from poultry to humans. Moreover, the dissemination of H5N1 in wild birds in areas later observed to be affected with human cases may represent direct transmission to humans who come into contact with wild birds. In 1997, 18 persons in Hong Kong became clinically infected with an avian influenza A virus (H5N1) that had caused a highly pathogenic avian influenza (HPAI) in poultry in the same region. Six of the patients died with clinical signs of severe influenza *(3, 4, 5)*. After culling approximately 1.5 million birds at live bird markets in Hong Kong, no other human cases of infection with this virus were identified that year. The virus appeared to lack the ability to efficiently spread from person to person.

Bird-to-human transmission of avian influenza A virus resulting in clinical disease has since been described with increasing frequency. In Southeast Asia in 1999, infection with avian influenza A virus H9N2 and H5N1 caused a limited number of clinical human infections, and at least one person died *(6, 7)*. During a large HPAI outbreak among poultry in the Netherlands in 2003, in which more than 30 million chickens had to be culled, the causative HPAI virus (H7N7) also was identified in 86 humans who had handled affected poultry and in three of their family members *(8, 9)*. The virus was closely related to low pathogenic avian influenza (LPAI) viruses identified in wild ducks prior to the outbreak *(8)*. The infected humans suffered from conjunctivitis and/or influenza-like illness, but the infection also resulted in fatal pneumonia with acute respiratory distress syndrome in one person *(8, 9)*. Subsequently, human infections with avian influenza A viruses (H7N2 and H7N3) occurred in the United States in 2003 and in Canada in 2004, resulting in one and two clinical cases, respectively *(6, 10, 11)*. Since December 2003, a rapidly increasing number of human infections with an avian influenza A virus (H5N1) have been identified in Southeast Asia, where direct or indirect contact with infected poultry and their excreta were the most likely source of infection in most, if not all, the cases. In Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey, and Vietnam, more than 200 human cases of infection with this virus have been identified, with a case fatality rate

higher than 50% (for an update, visit www.who.int/csr/disease/avian_influenza). Most of these infections were associated with respiratory disease, although diarrhea and neurological symptoms without severe respiratory disease also have been described in one or two patients *(12)*.

The pathogenicity of this H5N1 virus for different mammalian species upon experimental infection seems to have increased gradually over time *(13)*. Fatal infections in tigers and leopards fed with chicken carcasses have been reported; probable animal-to-animal transmission also has occurred in tigers *(14, 15)*. Experimental infection of domestic cats resulted in systemic spread of the virus, and animal-to-animal spread has been observed as well *(16, 17)*. In the first four months of 2006, the influenza A virus (H5N1) spread westward through Asia, probably with migratory birds, and reached the European Union. Wild and/or domestic birds became infected in 54 countries (for an update, visit http://www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm). In Turkey, where the virus caused extensive outbreaks of HPAI in poultry, 12 people became clinically infected after direct or indirect contacts with affected poultry; four died.

The crucial question today is whether these ongoing zoonotic events of the past decade increase the risk of the emergence of an influenza pandemic in humans. Until 1997, it was generally believed that the main risk involved the simultaneous infection of a mammalian species, such as the pig, with a human and an avian influenza A virus. This could then result in the emergence of a reassortant virus that could efficiently spread among humans in the virtual absence of pre-existing specific immunity in the human population at large. In fact, the "Asian flu" and "Hong Kong flu" pandemics were caused by viruses that were reassortants between avian and mammalian influenza A viruses. Direct infection of humans by avian influenza A viruses, as has been seen extensively since 1997, would create the possibility that such reassortant viruses could directly emerge in humans, if such infections occurred during episodes of epidemic influenza in humans.

A second scenario that could lead to the emergence of a pandemic influenza virus would be if an avian influenza A virus infected humans and gradually adapted to humans by sequential mutation, which could then open the door to efficient human-to-human transmission. The "Spanish flu" pandemic virus was probably not a result of a reassortment event; the virus probably adapted to humans by sequential mutation, although it is unknown whether other mammalian species were involved *(18, 19)*. It is currently difficult to predict whether the ongoing influenza A virus (H5N1) infections in humans in Eurasia will lead to the next influenza pandemic. However, even if they do not, it is important to consider the urgency of having in place early warning systems and pandemic preparedness plans to cope with such an event.

Several countries have stepped up their efforts by creating national preparedness committees that have drafted and put in place national plans. Nonetheless, much work remains to be done for countries to be adequately prepared.

VACCINES FOR PANDEMIC INFLUENZA: WHERE DO THEY STAND?

In 1999, the World Health Organization (WHO) developed the first comprehensive staged plan for responding to a pandemic influenza threat. In the past, work primarily had been based on human virological surveillance activities for epidemic influenza, in which a national influenza center and WHO collaborating centers have participated actively for more than half a century. The program has been updated continuously, and recently led to the development of the WHO Global Agenda on Influenza (www.who.int/influenza), whose mission was expanded from surveillance to pandemic preparedness, assessment of the impact of influenza, and increased influenza vaccine usage. The cornerstone of pandemic influenza preparedness is the ability to rapidly produce and distribute a specific pandemic vaccine. Given the lead time required to develop and produce such a vaccine, it will certainly not be available for distribution during the first six months of a pandemic outbreak. Therefore, to bridge the gap between the onset of the outbreak and the initial pandemic vaccine distribution, stockpiles of antiviral drugs may be an important adjunct in the efforts to reduce the spread of the virus, as well as morbidity and mortality in this period. Mainly due to pre-existing or rapidly developing antiviral resistance, the oldest anti-influenza drugs—the adamantanes—will probably be of little use. New generation anti-influenza drugs—the neuraminidase inhibitors (NIs)—are, therefore, probably the drugs of choice. Because these drugs may develop antiviral resistance when used extensively, the use of combinations of different groups of antiviral drugs may be advisable *(20)*. Furthermore, it should be kept in mind that the current global production capacity for the NIs will only allow production to cover therapeutic use for 1%–2% of the world's population. License agreement between the current NI producing companies and other companies elsewhere in the world may help lessen supply problems.

Production, distribution capacity, and efficacy also are key issues of pandemic influenza vaccines. Current epidemic or inter-pandemic influenza vaccines are predominantly inactivated subunit—split—or whole-virus vaccines, although recently cold-adapted live attenuated vaccines (CAIV-T) also have been introduced *(21)*. All these vaccines are still produced with embryonated chicken eggs as the production substrate, which

greatly limits the flexibility of production capacity. Therefore, the recent advent of cell-culture systems as a substrate, using continuous cell lines like MDCK and Vero cells, is considered to be a great improvement (22, 23). This advance will create a continuous availability of production capacity with great possibilities for further improvement and optimization of production processes. Several commercial companies are now focusing on these technologies, and the first vaccines produced by cell culture may soon be available.

The rapid generation of vaccine seed strains is another area that may help reduce lead time. Today, seed viruses are produced by WHO collaborating centers when WHO recommends an antigenically new epidemic influenza virus strain for inclusion in the inter-pandemic vaccine. Classically, these vaccine seed strains are produced by double infection of embryonated chicken eggs, using the recommended virus strain and the laboratory strain PR8 (which grows to high titers in these eggs), in order to produce a high growth reassortment. The use of reverse genetics for this purpose offers several advantages over the classical reassortment approach: it is a more rational and direct approach, it saves time, and it solves the problem of the possible presence of advantageous viruses in the epidemic virus isolate that could eventually contaminate the vaccine seed strain. Finally, it offers the opportunity to modify the HA at the plasmid stage to remove pathogenic traits, like a basic cleavage site. The latter may be performed by replacing the basic cleavage site from a HPAI virus with that of a LPAI virus. A high-throughput virus backbone may be adjusted to a cell line validated for vaccine production like MDCK or Vero cells under quality-controlled conditions (24, 25). Reverse genetics also may play a role in the generation of increased virus or HA yields in such new cell substrates.

The process of generating vaccine seed strains for pandemic vaccines by reassortment or reverse genetics may be bypassed by directly using LPAI ancestor or related viruses from wild bird surveillance activities (26, 27). Alternatively, using reverse genetics, the whole HA from such a related LPAI virus may be used to directly construct a LPAI seed strain using a high throughput virus backbone (27). Therefore, ongoing surveillance programs for wild birds, which are important as an early warning system for the emergence of HPAI, may also lead to the generation of repertoires of LPAI viruses related to possible future pandemic human influenza viruses. Viruses from such repositories can then be used directly for the rapid development of vaccine seed strains. A prerequisite for this approach is that ongoing and extended adequate analyses of antigenic properties of such LPAI viruses from both Eurasian and American lineages be carried out in such a way that they eventually allow the selection of prototype vaccine seed strains with the matching antigenic properties. This

may be accomplished using the principles of recently published antigenic cartography studies based on multidimensional scaling algorithms *(28)*.

The inactivated, inter-pandemic influenza vaccines currently in use are based predominantly on the principle of inducing virus-neutralizing (and HA-inhibiting) antibodies directed against the HA of the virus. For example, the use of vaccines consisting of only HA, produced as recombinant protein expressed by highly efficient alternative production systems like baculovirus systems, is also being considered for epidemic and pandemic influenza vaccines *(29)*. Little attention is being paid to the contributory role of the NA of the virus in this regard. Given that only 9 NA subtypes have been identified, versus 16 HA subtypes of influenza A viruses, and that the NA is probably also less subjected to antigenic drift than the HA, efforts should be directed to better understand the potential of NA as an immunogen. This can also induce virus-neutralizing antibodies. When repositories of potential pandemic virus seed strains are being prepared, the potential of the NA to induce more broadly protective immune responses deserves further attention. A third influenza A virus protein that may elicit protective antibody responses is the M2 protein. M2 is minimally immunogenic upon natural infection and conventional vaccination, which may explain its relative conservation among human influenza A viruses. However, it has been documented that the external domain of this protein (M2e), when linked to an appropriate carrier such as hepatitis B viral core particles, becomes highly immunogenic, inducing antibodies that may protect mice against lethal influenza virus challenge *(30)*. Although these results have not been confirmed by some groups of investigators, whose studies only showed weak protection-mediated antibody-dependent NK cell activity *(31)*, other investigators have shown exacerbated disease in pigs after challenge with this approach *(32)*. Additional studies are needed, because they may lead to more broadly protective vaccines that could protect against emerging pandemic influenza viruses.

The correlates of protection against influenza virus infection or disease are still poorly understood. In addition to virus-neutralizing antibodies directed against the HA, the NA, or M2e, it is not known to what extent cell-mediated immunity plays a protective role. Cell-mediated immunity may be directed to proteins other than the surface glycoproteins, such as the more conserved regions of the internal proteins, thus providing broad cross-reactive immunity between different virus subtypes. So far, limited work has been done in this area that may eventually contribute to the development of broader cross-reactive vaccines. In principle, the new generation of live attenuated CAIV may be expected to induce cytotoxic T cell (CTL) mediated immunity similar to natural infection. However, CAIV-T vaccines are based on the so-called 2–6 reverse genetics system, in which only the HA and the NA are expressed on a high-throughput backbone. Thus, CTL responses generated to the internal proteins of the CAIV may

not cross-react with those of emerging pandemic viruses. Using classical non-adjuvanted formulations of inactivated vaccines for prototype pandemic vaccines in preclinical studies, and recently also in clinical trials, it was shown that multiple injections, even with high antigen concentrations, failed to induce virus-neutralizing antibody levels that were protective in animal models or that may be protective in humans *(33, 34)*. Consequently, human trials with adjuvanted prototype pandemic vaccines should be carried out immediately to demonstrate their efficacy with regard to their ability to induce adequate levels of virus-neutralizing antibody, as well as to determine their safety. The limited numbers of human trials carried out so far with alum or MF59 adjuvanted prototype vaccines have shown that at least two injections should be given with relatively high concentrations of HA. Both for antigen sparing strategies and for the reduction of the number of vaccine injections needed to induce protective immunity, additional human trials with other adjuvants should be carried out as soon as possible (Table 1).

GLOBAL INFLUENZA-VACCINE SUPPLY

The development, production, and worldwide distribution of pandemic influenza vaccines pose major problems. The first priority for producing

TABLE 1. Main opportunities for improving pandemic influenza vaccines, current scenarios, and likely improvements in the future.

Opportunities	Current scenario	Future improvements
Strain selection	Human surveillance	• Antigenic mapping techniques • Bird surveillance (repositories)
Seed-strain production	Classical reassortment	• Reverse genetics
Production substrate	Embryonated hen's eggs	• Continuous cell lines • Recombinant HA (and other) proteins (e.g., baculovirus system)
Vaccination targets based on correlates of protection	HA proteins	• N proteins • M2 proteins • Cell-mediated immunity • Mucosal immunity
Adjuvants for inactivated vaccines	Unadjuvanted (exception: MF59)	• Aluminium salts • MF59 • Virosomes • Iscoms • Others

a pandemic vaccine is the prompt development of vaccine seed strains, using state-of-the-art technology with available virus strains. Issues ranging from intellectual property rights, to novel technology such as reverse genetics, to virus strains, to production technology using continuous cell lines, for example, should be solved in the inter-pandemic period. It is not absolutely clear at this juncture which inactivated-vaccine formulation should be used, nor with which adjuvant, antigen concentration, or number of injections to provide safe and effective protection against a newly emerging pandemic influenza virus. As already said, human vaccine trials to demonstrate safety and efficacy of prototype pandemic vaccines should be carried out as soon as possible to solve these problems. Inter-pandemic influenza vaccines are unique from a licensing point of view, since the licensing process includes a procedure for rapid annual updates of vaccine strains *(24)*. In the event of an influenza pandemic, regulatory authorities also should anticipate a rapid licensing process of new vaccines. Moreover, national agencies should make arrangements to compensate vaccine producers in case liability claims are filed against them.

Vaccine-production capacity that relies on currently available technology using embryonated chicken eggs definitely will not be able to produce sufficient pandemic vaccines for the world's needs *(35)*. Although the use of inter-pandemic influenza vaccine is on the rise, especially in less developed countries, 60%–70% of the world's influenza vaccine is currently being produced in Europe. The best pandemic preparedness in terms of vaccine production capacity and distribution is an increased use of inter-pandemic vaccine. For this reason, Canada has considerably increased its domestic inter-pandemic vaccine production and use *(36)* and the European Scientific Working Group on Influenza (ESWI) has advocated an increase of the annual epidemic vaccination coverage to one-third of the population in Europe (www.eswi.org). It also is important to state here that preparedness planning for an influenza pandemic is not a public health priority for many developing countries; consequently, inter-pandemic vaccination coverage in these countries is low. Equitable distribution of pandemic influenza vaccine throughout the world is, therefore, a key issue that also should be addressed urgently (Box 1).

STRATEGIC PLAN CONSIDERATIONS: ESTABLISHING INFLUENZA TASK FORCES

Preparedness plans for an influenza pandemic should be developed, continuously updated, and tested by all national agencies responsible for public health, following recommendations included in WHO's Global Agenda on Influenza (www.who.int/influenza). To ensure that every country in

> **BOX 1.** Key issues to be resolved in the inter-pandemic phase if vaccines are to be quickly produced and distributed worldwide during a pandemic.
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> - Vaccine seed virus strains must be developed in a timely way, on the basis of surveillance data and using state-of-the-art-technology.
> - Safe and effective prototype pandemic influenza vaccines should be identified in human clinical trials, with special attention given to adjuvants for inactivated vaccines that allow the induction of protective immunity, preferably with one injection and with minimal antigen contents of the vaccines.
> - Fast registration and licensing procedures of candidate pandemic influenza vaccines must be put in place by regulatory authorities, allowing for a rapid global use of a pandemic vaccine.
> - Problems with intellectual property rights associated with novel vaccine development and production technology must be resolved.
> - Compensation for liability claims must be set up for vaccine developers.
> - Use of inter-pandemic influenza vaccine should be increased to levels that would allow for the production and global distribution of pandemic influenza vaccines.
> - Problems related to the equitable and timely global distribution of pandemic influenza vaccines must be resolved.

the world is fully prepared for the next influenza pandemic, efforts in this area by the responsible national agencies should be stepped up drastically. Because influenza pandemics, like most virus infections that threaten human health, originate in animal reservoirs, a pandemic outbreak response will require the involvement of many disciplines. To fully understand the global threat posed by avian influenza, well-coordinated investigations of influenza viruses in wild birds and poultry populations should be an essential part of the global pandemic preparedness agenda *(37)*.

The spread of severe acute respiratory syndrome (SARS) is yet another recent, global public health threat by a virus infection that spilled over from an animal reservoir. SARS originated in Asia and rapidly spread to many countries in the world, infecting about 8,000 people, of whom about 800 died. WHO's role in the response to this outbreak was exemplary. The Organization constituted expert teams to rapidly respond to this emerging global health threat, one of which was the WHO SARS etiology team. That team consisted of laboratories in the region where the outbreak originated and laboratories with specific expertise in the area of emerging infectious diseases in other places of the world. This coordinated response resulted in the rapid identification and characterization of the etiological agent— SARS coronavirus (SARS CoV)—and the development of effective intervention strategies in just a couple of weeks *(38–41)*. Although the epidemiological features of influenza viruses are quite different from those of the

SARS-CoV, this experience showed that global interdisciplinary collaboration under the leadership of a UN organization such as WHO should definitely be considered key to combat an emerging influenza pandemic.

On September 30, 2005, UN Secretary General Kofi Anan announced the appointment of a United Nations system coordinator for pandemic influenza. This new appointment is designed to coordinate relevant agencies within the UN system, both to guide the centralized response to such an event and to provide support to Member States in this effort. Priority activities that are being promoted include early viral detection of influenza viruses in wild and domestic birds and in other animal species as a first line of defense against pandemic influenza. Surveillance in humans should continue to rely on WHO's influenza surveillance network, but should also enhance such surveillance so as to comply with the more sensitive requirements of the Organization's newly adopted International Health Regulations (IHR-2005). The initiative also will provide support to Member States in their efforts to develop national influenza pandemic preparedness plans, especially in developing countries.

Although the containment of a pandemic has never been attempted before, encouraging models have recently emerged. Two groups have demonstrated that with adequate early detection of human-to-human transmission it may be possible to halt an influenza pandemic in its earliest stages through targeted mass prophylactic use of antiviral drugs and the adoption of non-pharmaceutical interventions *(42)*. In order to make such an approach viable, early detection and rapid outbreak response systems must be in place in every country in accordance with WHO guidelines and in coordination with other specialized UN agencies, such as the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO).

INFLUENZA PREPAREDNESS IN THE AMERICAS

During the Presidential Summit of the Americas in Mar del Plata, Argentina, in November 2005, the Region's countries committed themselves, with the support of the Pan American Health Organization (PAHO), to completing their national plans to face the potential threat posed by the current outbreak of influenza H5N1 (see Table 2 for the status of these preparedness plans). Before this commitment had been made, PAHO had established an interprogrammatic and multidisciplinary task force on epidemic alert and response (the EAR Task Force) to meet the increased demand for technical cooperation necessitated by the emergence of an influenza strain with pandemic potential. The EAR Task Force has been charged with advising, coordinating, and monitoring all PAHO activities related to the planning and implementation of influenza pandemic pre-

TABLE 2. Status of national influenza pandemic preparedness plans, Region of the Americas, as of May 16, 2006.

Country	Ongoing preparedness activities	Draft plan received by PAHO	Plan published or available on the Internet	Plan endorsed by country authorities
Antigua and Barbuda	YES	NO	NO	NO
Argentina	YES	YES	YES	YES
Bahamas	YES	NO	NO	NO
Barbados	YES	YES	NO	NO
Belize	YES	YES	NO	NO
Bolivia	YES	YES	YES	YES
Brazil	YES	YES	YES	YES
Canada	YES	YES	YES	YES
Chile	YES	YES	YES	YES
Colombia	YES	YES	YES	YES
Costa Rica	YES	YES	NO	YES
Cuba	YES	YES	NO	YES
Dominica	YES	YES	NO	NO
Dominican Republic	YES	YES	NO	NO
Ecuador	YES	YES	YES	YES
El Salvador	YES	YES	NO	NO
Grenada	YES	NO	NO	NO
Guatemala	YES	YES	NO	NO
Guyana	YES	YES	NO	NO
Haiti	YES	NO	NO	NO
Honduras	YES	YES	NO	NO
Jamaica	YES	NO	NO	NO
Mexico	YES	YES	YES	YES
Nicaragua	YES	YES	NO	YES
Panama	YES	YES	YES	YES
Paraguay	YES	YES	NO	NO
Peru	YES	YES	YES	YES
Puerto Rico	YES	YES	NO	NO
Saint Kitts and Nevis	YES	NO	NO	NO
Saint Lucia	YES	NO	NO	NO
Saint Vincent and the Grenadines	YES	YES	NO	NO
Suriname	YES	YES	NO	NO
Trinidad and Tobago	YES	YES	NO	NO
United States of America	YES	YES	YES	YES
Uruguay	YES	YES	YES	YES
Venezuela	YES	YES	YES	NO

Source: Immunization Unit, Pan American Health Organization.

paredness and response. All EAR Task Force activities are framed under the new mandates set forth in WHO's International Health Regulations 2005 (IHR-2005), which stipulate that countries should develop, strengthen, and maintain core capacities to detect, assess, and intervene rapidly to control

events of international public health importance related to risk or disease. The task force's interprogrammatic nature responds to the complex process involved in the implementation and influenza pandemic planning contemplated in IHR-2005. This work also requires that a variety of sectors, including the private sector, participate in highly coordinated efforts.

Under EAR Task Force's interprogrammatic framework, technical cooperation in influenza preparedness has included providing support to Member States to develop their national influenza pandemic preparedness plans (NIPPPs). PAHO has distributed multi-language guidelines to assist in the effort. Subregional workshops using modeling software have been conducted to estimate the potential impact of a pandemic based on multiple scenarios. The results of the modeling exercises have helped ensure that the countries' plans are flexible and can respond to many contingencies, including a worst-case scenario where there are neither available vaccines nor antiviral medications. This planning also highlights the need for the NIPPPs to prioritize interventions and address other important issues, such as access to health care.

PAHO has developed an assessment tool, based on WHO's checklist for influenza preparedness (http://www.who.int/csr/resources/publications/influenza/FluCheck6web.pdf), to assess national plans. Assessment exercises with multidisciplinary country delegations allow for comprehensive self-assessments to be made of national influenza pandemic preparedness plans and for the exchange of ideas and strategies between countries. Important lessons learned have highlighted the need to address chain-of-command and coordination issues that may be encountered during a pandemic or during the pandemic alert period. In the Americas, countries also have conducted simulation exercises. Based on them, action plans should be developed aimed at filling the gaps identified by the self-assessments and by the simulations. Further multisectoral collaboration in the refinement of such plans will be needed.

PAHO also supports its Member States in operationalizing national influenza preparedness plans at the local level, to ensure an effective response to a pandemic. To this end, pilot interventions have been carried out in selected countries as a way to harmonize the local implementation of national plans, thus ensuring that communities at the front line of a possible pandemic will be prepared.

References

1. Potter CW. Chronicles of influenza pandemics. In: Nicholson KG, Webster RG, Hay AJ, eds. *Textbook of influenza*. Malden, MA: Blackwell Publishing, 1998: 3–18.
2. Fouchier RA, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, Rimmelzwaan GF, Olsen B, Osterhaus AD. Characterization of a novel in-

fluenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Viral* 2005;79(5):2814–2822.

3. de Jong JC, Claas EC, Osterhaus AD, Webster RG, Lim WL. A pandemic warning? *Nature* 1997;389(6651):554.

4. Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, Krauss S, Shortridge KF, Webster RG. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet.* 1998 Feb 14;351 (9101):472–477. Erratum in: *Lancet* 1998;351(9111):1292.

5. Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, Perdue M, Swayne D, Bender C, Huang J, Hemphill M, Rowe T, Shaw M, Xu X, Fukuda K, Cox N. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 1998;279(5349):393–396.

6. Lin YP, Shaw M, Gregory V, Cameron K, Lim W, Klimov A, Subbarao K, Guan Y, Krauss S, Shortridge K, Webster R, Cox N, Hay A. Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. *Proc Natl Acad Sci U S A* 2000;97(17):9654–9658.

7. Peiris M, Yuen KY, Leung CW, Chan KH, Ip PL, Lai RW, Orr WK, Shortridge KF. Human infection with influenza H9N2. *Lancet* 1999;354(9182):916–917.

8. Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, Kuiken T, Rimmelzwaan GF, Schutten M, Van Doornum GJ, Koch G, Bosman A, Koopmans M, Osterhaus AD. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A* 2004;101(5):1356–1361.

9. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, Meijer A, van Steenbergen J, Fouchier R, Osterhaus A, Bosman A. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 2004;363(9409): 587–593.

10. Olofsson S, Kumlin U, Dimock K, Arnberg N. Avian influenza and sialic acid receptors: more than meets the eye? *Lancet Infect Dis* 2005;5(3):184–188.

11. Hirst M, Astell CR, Griffith M, Coughlin SM, Moksa M, Zeng T, et al. Novel avian influenza H7N3 strain outbreak, British Columbia. *Emerg Infect Dis* 2004;10(12):2192–2195.

12. de Jong MD, Bach VC, Phan TQ, Vo MH, Tran TT, Nguyen BH, Beld M, Le TP, Truong HK, Nguyen VV, Tran TH, Do QH, Farrar J. *N Engl J Med* 2005;352(7): 686–691.

13. Maines TR, Lu XH, Erb SM, Edwards L, Guarner J, Greer PW, Nguyen DC, Szretter KJ, Chen LM, Thawatsupha P, Chittaganpitch M, Waicharoen S, Nguyen DT, Nguyen T, Nguyen HH, Kim JH, Hoang LT, Kang C, Phuong LS, Lim W, Zaki S, Donis RO, Cox NJ, Katz JM, Tumpey TM. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *J Virol* 2005;79(18):11788–11800.

14. Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amonsin A, Payungporn S, Noppornpanth S, Wattanodorn S, Theamboonies A, Tantilertcharoen R, Pattanarangsan R, Arya N, Ratanakorn P, Osterhaus DM, Poovorawan Y. Avian influenza H5N1 in tigers and leopards. *Emerg Infect Dis* 2004;10(12): 2189–2191.

15. Thanawongnuwech R, Amonsin A, Tantilertcharoen R, Damrongwatanapokin S, Theamboonlers A, Payungporn S, Nanthapornphiphat K, Ratanamungk-

lanon S, Tunak E, Songserm T, Vivatthanavanich V, Lekdumrongsak T, Kesdangsakonwut S, Tunhikorn S, Poovorawan Y. Probable tiger-to-tiger transmission of avian influenza H5N1. *Emerg Infect Dis* 2005;11(5):699–701. Erratum in: *Emerg Infect Dis* 2005;11(6):976.

16. Kuiken T, Rimmelzwaan G, van Riel D, van Amerongen G, Baars M, Fouchier R, Osterhaus A. Avian H5N1 influenza in cats. *Science* 2004;306(5694):241. Epub 2004 Sep 2.

17. Rimmelzwaan GF, van Riel D, Baars M, Bestebroer TM, van Amerongen G, Fouchier RA, Osterhaus AD, Kuiken T. Influenza A virus (H5N1) infection in cats causes systemic disease with potential novel routes of virus spread within and between hosts. *Am J Pathol* 2006;168(1):176–183.

18. Taubenberger JK, Reid AH, Krafft AE, Bijwaard KE, Fanning TG. Initial genetic characterization of the 1918 "Spanish" influenza virus. *Science* 1997;275 (5307):1793–1796.

19. Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solorzano A, Swayne DE, et al. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 2005;310(5745):77–80.

20. de Jong MD, Tran TT, Truong HK, Vo MH, Smith GJ, Nguyen VC, et al. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N Engl J Med* 2005;353(25):2667–2672.

21. Belshe RB. Current status of live attenuated influenza virus vaccine in the US. *Virus Res*. 2004;103(1–2):177–185.

22. Brands R, Visser J, Medema J, Palache AM, van Scharrenburg GJ. Influvac: a safe Madin Darby Canine Kidney (MDCK) cell culture-based influenza vaccine. *Dev Biol Stand* 1999;98:93–100; discussion 111.

23. Oxford JS, Manuguerra C, Kistner O, Linde A, Kunze M, Lange W, et al. A new European perspective of influenza pandemic planning with a particular focus on the role of mammalian cell culture vaccines. *Vaccine* 2005;23(46–47): 5440–5449.

24. Nicolson C, Major D, Wood JM, Robertson JS.Generation of influenza vaccine viruses on Vero cells by reverse genetics: an H5N1 candidate vaccine strain produced under a quality system. *Vaccine* 2005;23(22):2943–2952.

25. Webby RJ, Perez DR, Coleman JS, Guan Y, Knight JH, Govorkova EA, McClain-Moss LR, Peiris JS, Rehg JE, Tuomanen EI, Webster RG. Responsiveness to a pandemic alert: use of reverse genetics for rapid development of influenza vaccines. *Lancet* 2004;363(9415):1099–1103.

26. Munster VJ, Wallensten A, Baas C, Rimmelzwaan GF, Schutten M, Olsen B, Osterhaus AD, Fouchier RA. Mallards and highly pathogenic avian influenza ancestral viruses, northern Europe. *Emerg Infect Dis* 2005;11(10):1545–1551.

27. de Wit E, Munster VJ, Spronken MI, Bestebroer TM, Baas C, Beyer WE, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. Protection of mice against lethal infection with highly pathogenic H7N7 influenza A virus by using a recombinant low-pathogenicity vaccine strain. *J Virol* 2005;79(19):12401–12407.

28. Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. Mapping the antigenic and genetic evolution of influenza virus. *Science* 2004;305(5682):371–376. Epub 2004 Jun 24.

29. Fiers W, De Filette M, Birkett A, Neirynck S, Min Jou W. A "universal" human influenza A vaccine. *Virus Res* 2004;103(1–2):173–176. Review.

30. Jegerlehner A, Schmitz N, Storni T, Bachmann MF. Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. *J Immunol* 2004;172(9):5598–5605.

31. Heinen PP, Rijsewijk FA, de Boer-Luijtze EA, Bianchi AT. Vaccination of pigs with a DNA construct expressing an influenza virus M2-nucleoprotein fusion protein exacerbates disease after challenge with influenza A virus. *J Gen Virol* 2002;83(Pt 8):1851–1859.

32. Rimmelzwaan GF, Claas EC, van Amerongen G, de Jong JC, Osterhaus AD. ISCOM vaccine induced protection against a lethal challenge with a human H5N1 influenza virus. *Vaccine* 1999;17(11–12):1355–1358.

33. Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, Zambon MC, Katz JM. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. *J Infect Dis* 2005;191(8):1210–1215. Epub 2005 Mar 14.

34. Fedson DS. Vaccination for pandemic influenza: a six point agenda for interpandemic years. *Pediatr Infect Dis J* 2004;23(1 suppl):S74–S77.

35. Tam T, Sciberras J, Mullington B, King A. Fortune favours the prepared mind: a national perspective on pandemic preparedness. *Can J Public Health* 2005 Nov-Dec;96(6):406–408.

36. Fouchier R, Kuiken T, Rimmelzwaan G, Osterhaus A. Global task force for influenza. *Nature* 2005;435(7041):419–420.

37. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, Nicholls J, Yee WK, Yan WW, Cheung MT, Cheng VC, Chan KH, Tsang DN, Yung RW, Ng TK, Yuen KY, SARS Study Group. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003;361(9366):1319–1325.

38. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003;348(20):1967–1976. Epub 2003 Apr 10.

39. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ, SARS Working Group. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;348(20):1953–1966. Epub 2003 Apr 10.

40. Fouchier RA, Kuiken T, Schutten M, van Amerongen G, van Doornum GJ, van den Hoogen BG, Peiris M, Lim W, Stohr K, Osterhaus AD. Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* 2003;423(6937):240.

41. Ferguson NM, Cummings DA, Cauchemez S, Fraser C, Riley S, Meeyai A, Iamsirithaworn S, Burke DS. Strategies for containing an emerging influenza pandemic in Southeast Asia. *Nature* 2005;437(7056):209–214. Epub 2005 Aug 3.

42. Longini IM Jr, Nizam A, Xu S, Ungchusak K, Hanshaoworakul W, Cummings DA, Halloran ME. Containing pandemic influenza at the source. *Science* 2005;309(5737):1083–1087. Epub 2005 Aug 3.