



# H5N1 Influenza Continues To Circulate and Change

As the H5N1 viruses continue to expand their range and behave in unexpected ways, they remain a serious threat to birds and humans

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

**T**he spread of the highly pathogenic H5N1 influenza virus from eastern Asia to India, Europe, and Africa raises concern that it will become endemic in wild birds throughout the world. Wild aquatic birds are reservoirs for all 16 subtypes of influenza virus, many of which rarely harm these hosts. However, the H5N1 virus that emerged in southern China in 1996 and then was transmitted to humans (Fig. 1) readily killed waterfowl in Penfold and Kowloon Parks in Hong Kong. The next H5N1 viruses that were isolated in Asia in 2003 proved sporadically lethal to waterfowl, but were

100% lethal for poultry such as chicken, quail, and turkey.

Meanwhile, that 1996 avian H5N1 virus split into two distinguishable lineages, one found in Vietnam and Thailand and the other in Indonesia, by early 2004. These new lineages spread mainly through transport of domestic poultry rather than by wild, migratory birds. The second major spread of this H5N1 virus occurred following the emergence of the A/Bar-headed (BH) Goose/Qinghai/65/05 (H5N1) influenza virus in May 2005, whose gene components trace to H5N1 viruses that were isolated earlier from mallard ducks at Poyang Lake in southwest China. The Poyang Lake viruses contributed gene segments to the Qinghai H5N1 virus that are phylogenetically closely related to H5N1 viruses isolated in domestic poultry (A/Chicken/Shantou/423/03 [H5N1]).

Although migratory birds likely account for much of the remarkably rapid spread of H5N1 viruses from Qinghai Lake in western China to Mongolia, Russia, and Turkey, humans also may have helped to spread the virus along rail lines. While the A/BH Goose/Qinghai/65/05 (H5N1) viruses are highly pathogenic to geese, they are nonlethal in mallard ducks. Thus, bar-headed geese can be ruled out as spreading this virus to Europe, whereas ducks remain a likely suspect, albeit without firm proof. Although deaths among swans and geese served as sentinel events for detecting H5N1 in Europe, these two species probably do not distribute H5N1 viruses over great distances.

## Summary

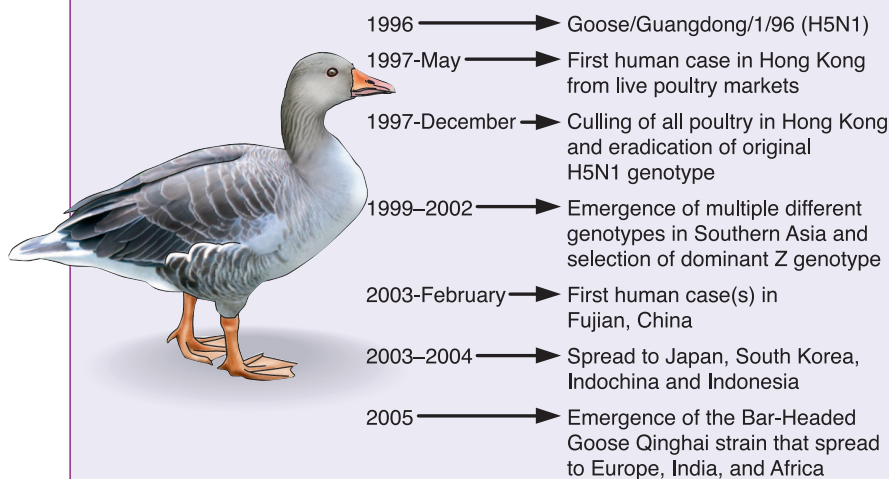
- Highly pathogenic variants of H5N1 influenza virus that moved from Asia to India, Europe, and Africa have continued to evolve in Southeast China, giving rise to novel variants and raising concerns that these viruses will spread elsewhere and become endemic in wild birds throughout the world.
- More than half of the 250 persons infected so far with H5N1 died, and efforts to control the spread of this virus led to culling of more than 200 million domestic birds in Asia.
- The H5N1 viruses are overturning several concepts about how influenza viruses behave, including ideas about what species of birds safely transmit them and which bird species the viruses can sicken and kill; several species of cat can be infected with and transmit H5N1.
- Once a particular influenza virus becomes widespread among bird species within a region, control measures that depend on culling of flocks need to be augmented with vaccine use.

*Robert Webster is a professor in the Division of Virology, Department of Infectious Diseases at St. Jude Children's Research Hospital, Memphis, Tenn.; Yi Guan is Professor and Director of the State Key Laboratory of Emerging Infectious Diseases Department of Microbiology, Malik Peiris is Professor, in the Department of Microbiology, and Honglin Chen is Assistant Professor, State Key Laboratory for Emerging Infectious Diseases in the Department of Microbiology at The University of Hong Kong, Hong Kong, China.*



FIGURE 1

### Genesis of H5N1 Influenza in Asia



Genesis of H5N1 influenza in Asia.

### Changing Patterns of H5N1 Viruses in Birds

The emergence of the highly pathogenic H5N1 influenza viruses in Asia in 1996 and their subsequent transmission to domestic poultry, humans, and other mammals, including felids, is unprecedented in the recorded history of influenza. These viruses continue to change. Remarkably, their unfolding story appears to violate ecological principles that were established for other influenza viruses during the past 30 years.

The newer viruses appear to spread readily as aerosols among birds, unlike influenza viruses that circulated in mallard ducks, for example, before 2002 (Fig. 2). Thus, based on 23 different H5N1 viruses in mallard ducks, the amount of virus excreted orally increased by a factor of 10 after 2002. These newer viruses replicate to higher titers in the upper respiratory tract than in the gastrointestinal tracts of their host ducks.

Although these strains of H5N1 will still be transmitted primarily through water in nature, they likely will be transmitted primarily by the respiratory route within facilities such as poultry markets. To track outbreaks, investigators will need to collect oral, tracheal, and water trough samples as well as cloacal/fecal samples.

Before 2002, all 16 subtypes of nonpatho-

genic influenza viruses and the vast majority of highly pathogenic H5 and H7 influenza viruses caused no disease signs in waterfowl. However, the current H5N1 viruses are overturning this concept. Although not uniformly pathogenic, the lethality of the post-2002 H5N1 viruses varies with the specific virus and species and age of waterfowl. Thus, some H5N1 isolates are 100% lethal to mallard ducks, whereas others are not, enabling some ducks to spread H5N1. Because mallard ducks can shed nonlethal H5N1 viruses for up to 20 days, non-pathogenic variants likely have a selective advantage in these ducks.

Probably the first recorded incident of influenza virus moving from wild birds directly to humans occurred in Azerbaijan in

February 2006—apparently when individuals were collecting down and feathers from dead wild swans. However, because of a ban on handling dead birds, those involved were reluctant to admit to having handled the wild birds. Officials of the World Health Organization (WHO) confirmed eight human cases of H5N1 infection, five of whom died.

### H5N1 Viruses Move from Birds to Cats and to Other Species

The post-2001 H5N1 influenza strains also infect, are transmitted among, and kill tigers, leopards, and other felids, including domestic cats. The first noted outbreaks occurred at zoos in Thailand when tigers and leopards were fed infected chickens.

Domestic cats were first reported to be infected near Bangkok, Thailand, in February 2004, when 14 of 15 cats died after one of them ate an infected chicken carcass. Although the virus can replicate outside the respiratory tract, titers are highest in lungs. The virus also infected domestic cats in Iraq, Indonesia, and Germany that consumed infected wild birds. H5N1 viruses apparently also infected masked palm civets (*Paguma larvata*) in Vietnam, a dog in Thai-

land, and a stone marten (*Mustelidae* family) in Germany.

Although H5N1 viruses are poorly transmitted from poultry to humans, the viruses move freely from poultry to cats and then among cats—raising the possibility that cats will serve as an intermediate host that promotes transmission to humans and transmission from human to human.

### Stability and Environmental Sources of Influenza Viruses

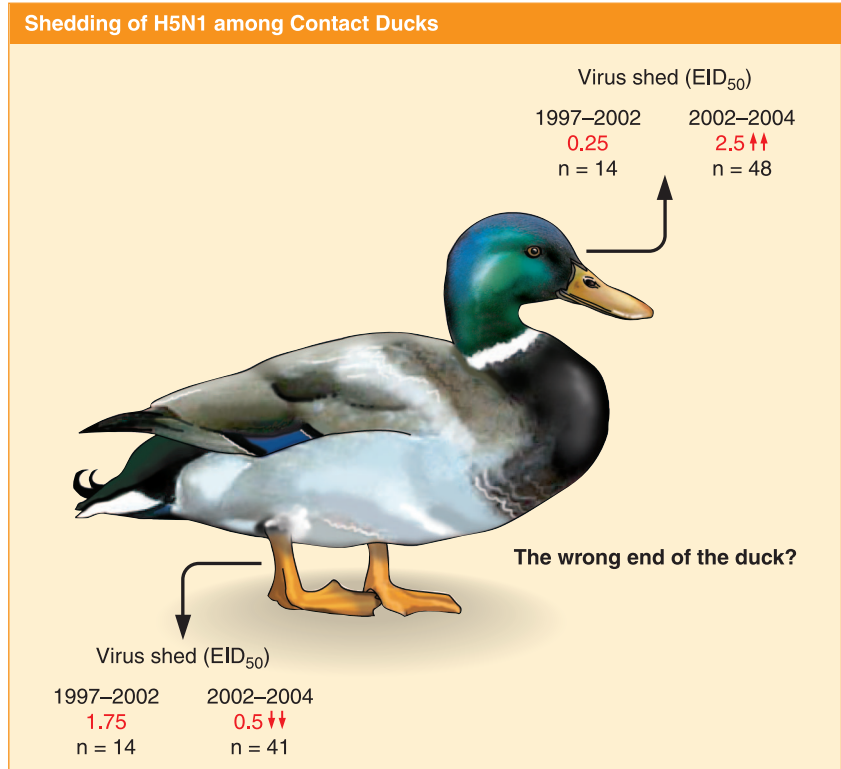
Another important property of avian influenza viruses is their remarkable stability in aqueous suspension. Many subtypes retain their infectivity for more than 100 days at 28°C when the initial concentration is  $10^6$  TCID<sub>50</sub>. Although the infectivity of the 1997 Hong Kong H5N1 virus lasted only 2 days at 37°C, aqueous suspensions of post-2001 H5N1 viruses remain infective for 4 to 6 days at 37°C. Other H5N1 variants circulating in Eurasia are also environmentally stable, providing additional opportunities for these viruses to transmit to people and poultry through untreated water.

Investigators formerly believed that influenza viruses are transmitted unidirectionally from wild migratory birds to domestic species. However, the A/BH Goose/Qinghai/65/05 (H5N1) virus is a reassortant that obtained its M, NS, PA, PB1, and PB2 gene segments from A/Mallard duck/Jiangxi/1653/05 (H5N1) and its HA, NA, and NP segments from A/Mallard duck/Jiangxi/2136/05 (H5N1). In both cases, domestic ducks infected mallards that were over-wintering in southern China.

Thus, the A/BH Goose/Qinghai/65/05 (H5N1) virus apparently contains gene segments from influenza viruses in domestic birds, along with gene segments that were spread in part by migratory birds. The A/BH Goose/Qinghai/65/05 (H5N1) virus contains lysine at residue 627 of PB2, which is associated with pathogenicity in mammals. Did the selection of the PB2 lysine 627 variant occur in mammals in China?

Since 2001, investigators report finding H5N1 infecting passerine birds, some of which were

FIGURE 2



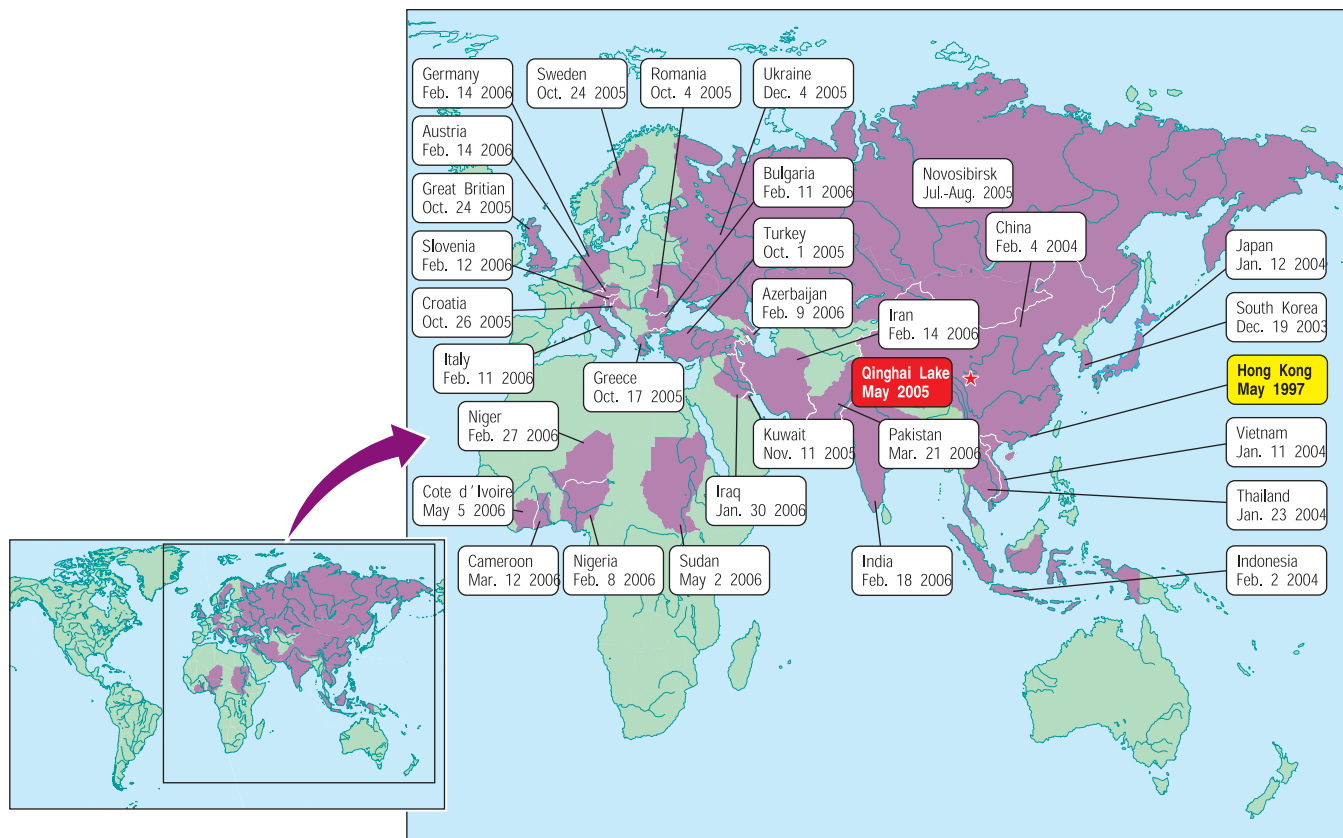
Which end of the bird? Ecological studies of influenza viruses in wild aquatic birds over a period of 30 years showed the viruses to replicate primarily in the cells lining the intestinal tract and to be shed in feces. Thus, virus was spread fecally-orally through water. The highly pathogenic Asian H5N1 virus that emerged after 2002 changed this pattern: virus was shed primarily from the respiratory tract. This finding suggests that the virus can spread in enclosed buildings and that tracheal swabbing must be included in ecological studies of the spread of this virus.

killed during the 2002–2003 outbreak in Hong Kong. Similarly, H5N1 viruses were isolated from dead crows near affected chicken farms in Japan.

Early in 2006, H5N1 influenza viruses were isolated from dead crested mynahs, common magpies, oriental magpie robins, and Japanese white-eyes in the New Territories of Hong Kong. At times, passerine birds seemed to be falling from the sky. These events received limited attention perhaps because they were taken to reflect infections in domestic poultry rather than establishment of H5N1 viruses in wild passerine populations. Although infections in domestic poultry were the probable source of these H5N1 viruses, passerine birds doubtless contribute to the spread of H5N1 viruses in nearby domestic poultry flocks and among cats.



FIGURE 3



Spread of H5N1 influenza in Eurasia and absence (to date) in the Americas and Australia. There have been three waves of H5N1 spread in Eurasia: First in mid 2003 – early 2004 in Eastern Asia; second in mid 2004–late 2004 with resurgence in Eastern Asia and China; third in December 2004/2006 spread through central Asia to Africa, Europe, and India (for details see ([www.who.int/influenza](http://www.who.int/influenza))). Note that the Americas and Australia are free of HP H5N1, despite the inclusion of Alaska as part of the east Asia–Australia flyway of migratory birds (the same flyway that includes Indonesia, Vietnam, Thailand, and eastern China).

### One Strategy for Controlling H5N1 Viruses Entails Eradicating Source Animals

Options for controlling influenza viruses include eradicating source animals, vaccinating animals that might become infected, and using antivirals. Here we consider eradication, vaccination, and combinations of those two approaches.

Developed countries typically eradicate domestic poultry flocks to control highly pathogenic (HP) avian influenza. Historically, several countries in the Americas and in Eurasia controlled HP H5 and H7 viruses by quarantine and culling flocks, introducing improved biosecurity, and then compensating growers. For example, this approach was used to rid H7N7 from Dutch poultry in 2004, H5N2 from Pennsylva-

nia poultry in the 1980s, and H7N3 from Canada in 2003. Similar approaches were leveled on H5N1 in Japan and South Korea after 2003 and in Thailand in 2005.

Such measures are costly in affluent countries. Although such countries can afford to eradicate highly pathogenic H5N1 influenza viruses by culling poultry, this option would impose too great a burden in developing countries, where backyard poultry is an important source of protein. Not only do eradication efforts have devastating economic and nutritional impacts, villagers are also reluctant to report outbreaks of infections in poultry.

Furthermore, while culling approaches may prove successful when domestic poultry flocks are targeted, this strategy is not workable when dealing with viruses that are circulating in wild-





bird reservoirs. Thus, recognizing that influenza is not truly an eradicable disease, influenza experts continue to be concerned that H5N1 viruses in Eurasia are becoming established in migratory birds. For instance, the H5N1 virus was reintroduced into domestic poultry in Thailand in July 2006 not long after it was supposedly eradicated there. Hence, once a particular influenza virus becomes widespread within a region, vaccine use becomes an essential component of control measures.

### **Controlling H5N1 Influenza also Entails Vaccinating Animals**

Despite the need for vaccines to control H5N1 influenza viruses at the source, there are concerns over selecting antigenic variants and producing effective vaccines, on relying on those vaccines without improving other biosecurity measures, and on choosing specific means for deploying vaccines. Amid these concerns, there is an ongoing need to produce high-quality, inexpensive, standardized poultry vaccines for use in aquatic birds such as ducks and geese as well as in commercial poultry, including chickens, quail, pheasants, and guinea fowl. Reverse genetics strategies make design of such vaccines possible. Meanwhile, both inactivated high-growth, whole-virus oil emulsion vaccine and live-attenuated recombinant vaccines expressing the H5 HA in fowl pox or Newcastle disease virus are being evaluated.

One control approach calls for deploying vaccines in conjunction with quarantines to control the outbreak, and planning at the outset to discontinue vaccine use once the virus is controlled. Part of this strategy entails using analytic methods to discriminate infected from vaccinated birds. This approach successfully controlled an outbreak during 2003 of H7N2 viruses in layer chicken farms in Connecticut. Stringent biosecurity measures were implemented after vaccine use was discontinued.

Another approach calls for continuing use of vaccines to control disease and virus spread. Discontinuing vaccine use does not appear to be an option if a highly pathogenic H5N1 virus becomes endemic in a particular region. Thus, vaccinations have continued in Hong Kong and at poultry farms in southern China that provide chickens to Hong Kong markets since 2004, with monitoring of unvaccinated chickens in

poultry houses to ensure that the vaccines prevent virus spread. Although this strategy prevented reintroduction of H5N1 virus into Hong Kong from legitimate imports, the virus came in via smuggled chickens and, by February 2006, infected wild waterfowl and passerine birds. These events led officials to ban backyard poultry in Hong Kong, enforced with a heavy monetary fine.

Despite improved methods for designing and making influenza vaccines for humans and poultry (mainly chickens), domestic ducks and geese, which are reservoir species, also need to be vaccinated. For instance, in 2004 and 2005, H5N1 virus was isolated from about 2% of apparently healthy geese and ducks every month in southern Chinese poultry markets.

The favored policy for controlling H5N1 influenza in China is to vaccinate all poultry. Despite residual H5N1 virus shedding from apparently healthy poultry flocks, the inoculated birds apparently are protected against disease, if not against viral replication. Failure to standardize the antigen content of agricultural vaccines is of continuing concern, and substandard agricultural vaccines are a continuing problem.

As part of an ongoing nationwide effort, officials in Vietnam began using vaccines to control H5N1 influenza in poultry in October 2005, deploying two rounds to vaccinate 170 million chickens and 79 million ducks in 64 provinces. Notably, since mid-November 2005, no cases of H5N1 infection in humans or chickens were reported from Vietnam, whereas earlier there were 93 human cases with 42 deaths. Surveillance reports also indicate no detectable H5N1 viruses in chickens but infections of ducks and geese. Although encouraging, further surveillance is needed, and monitoring of sentinel unvaccinated chickens is warranted. A recent report from Vietnam of H5N1 in ducks and in smuggled poultry indicates the continued presence of the virus.

### **Several Key Questions Linger**

Certainly there will be another influenza pandemic, but whether H5N1, H2, H9, H7, or another influenza virus will acquire this potential remains unknown. However, H5N1 viruses are continuing to expand their range, to change, and to behave in unexpected ways (see p. 552). Thus, it would be a fundamental mistake to



become complacent about H5N1. More than half of 256 persons infected with H5N1 died, and efforts to control the spread of this virus led to culling of more than 200 million domestic poultry.

The flyways of migratory birds overlap in Alaska, making it possible that infected birds from Eurasia will introduce H5N1 into the Americas. However, phylogenetic records show that influenza viruses in wild birds are separated into two distinct American and Eurasian clades. These differences suggest that the viruses and birds do not mix as often as we might expect (Fig. 3). Why not? The current intense virus surveillance of migratory birds may provide insights into whether the flyways truly overlap and how frequently such birds exchange influenza viruses.

After narcotics, the most common items smuggled into the U.S. are live birds for the pet trade. Thus, birds carrying Asian H5N1 viruses may be smuggled into the Americas, or frozen domestic birds may be illegally imported from Asia. For instance, 1,600 pounds of frozen ducks, geese, and chickens were recently imported from China into Troy, Mich., without surveillance for viruses. And no one can rule out the possibility of an H5N1-infected human bringing this virus into the Americas.

Other highly pathogenic H5 and H7 influenza strains did not become established in wild birds. However, the new H5N1 virus appears to be breaking other rules by replicating harmlessly in some species while killing others. However, early in 2006, birds that migrated from Africa to Europe did not carry detectable H5N1 virus despite that virus being found among domestic poultry in Nigeria, Niger, and elsewhere in Africa. Will migratory birds become infected in Europe later during 2006 and then bring the virus back to Africa?

The situation in north-central Asia and Siberia is of continuing concern; the die-off of bar-headed geese in 2005 and the recent die-off of more than 4,000 crested grebes (*Podiceps cristatus*) in June 2006 on the Mongolian-Russian border suggests that the HP H5N1 virus is in the Siberian breeding grounds of migratory waterfowl. A continuing controversy is whether the wild migratory birds in western Asia are the source of continuing outbreaks in domestic

poultry or vice versa. Agricultural authorities blame the wild birds, while wildlife biologists blame the domestic poultry.

Prospective virological and serological influenza virus surveillance in Laos failed to detect H5N1 virus in 2005, despite sampling more than 6,000 domestic ducks, chickens, and quail. Thus, although H5N1 virus caused lethal outbreaks in several provinces, it was not established within domestic poultry flocks. However, recent surveillance in the Lao People's Democratic Republic indicated that a phylogenetically distinguishable H5N1 virus was introduced early in 2006 and for a second time in July. Because there was no migratory bird activity at those times, the most likely source was imported domestic poultry.

The expanded geographical range of H5N1 influenza viruses through much of Eurasia and its presence in Africa along with HIV infections provide an opportunity for continuing evolution and transmission in human populations that are immunologically compromised. Although the H5N1 virus has been circulating now for a decade in poultry with occasional transmissions to humans, we believe that it would be premature to become complacent regarding the potential of this virus to transmit in humans. The apparent increase in the number of family clusters of H5N1 in Indonesia is of continuing concern. The key unanswered questions include:

- Will H5N1 acquire consistent human-to-human transmissibility and cause a pandemic?
- How soon will H5N1 spread from Asia to the Americas?
- Will H5N1 become endemic in wild bird reservoirs?

These viruses kill more than 50% of humans that they infect. One such virus infected a family cluster of eight persons in Indonesia, and included three sequential human-to-human transmissions. Fortunately, however, that particular cluster did not expand. Moreover, in humans, the H5N1 viruses remain poorly transmissible between individuals. In over 40 years of experience with influenza, the Asian H5N1 is the most virulent virus I have encountered (R.G.W.); if it does acquire consistent human-to-human transmissibility—it will likely be catastrophic.

## ACKNOWLEDGMENTS

We thank Carol Walsh for manuscript preparation and Sharon Naron for editorial assistance.

This work was supported by Public Health Service grants AI-95357 and CA-21765 and by the American Lebanese Syrian Associated Charities (ALSAC).

## SUGGESTED READING

Chen, H., G. J. Smith, K. S. Li, J. Wang, X. H. Fan, J. M. Rayner, D. Vijaykrishna, J. X. Zhang, L. J. Zhang, C. T. Guo, C. L. Cheung, K. M. Xu, L. Duan, K. Huang, K. Qin, Y. H. Leung, W. L. Wu, H. R. Lu, Y. Chen, N. S. Xia, T. S. Naipospos, K. Y. Yuen, S. S. Hassan, S. Bahri, T. D. Nguyen, R. G. Webster, J. S. Peiris, and Y. Guan. 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc. Natl. Acad. Sci. USA* 103:2845–2850. (Epub 10 February 2006.)

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

Maines, T. R., L. M. Chen, Y. Matsuoka, H. Chen, T. Rowe, J. Ortin, A. Falcon, N. T. Hien, Q. Mai le, E. R. Sedyaningsih, S. Harun, T. M. Tumpey, R. O. Donis, N. J. Cox, K. Subbarao, and J. M. Katz. 2006. Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. *Proc. Natl. Acad. Sci. USA* 103:12121–12126. (Epub 31 July 2006.)

Olsen, B., V. J. Munster, A. Wallensten, J. Waldenstrom, A. D. Osterhaus, and R. A. Fouchier. 2006. Global patterns of influenza A virus in wild birds. *Science* 312:384–388.

Palese, P., T. M. Tumpey, and A. Garcia-Sastre. 2006. What can we learn from reconstructing the extinct 1918 pandemic influenza virus? *Immunity* 24:121–124.

Russell, C. J., and R. G. Webster. 2005. The genesis of a pandemic influenza virus. *Cell* 123:368–371.

Shinya, K., M. Edina, S. Yamada, M. Ono, N. Kasai, and Y. Kawaoka. 2006. Avian flu: influenza virus receptors in the human airway. *Nature* 440:435–436.

Taubenberger, J. K., and D. M. Morens. 2006. 1918 Influenza: the mother of all pandemics. *Emerg. Infect. Dis.* 12:15–22.

Wong, S. S. Y., and K. Y. Yuen. 2006. Avian influenza virus infections in humans. *Chest* 121:156–168.



*There is something for everyone at the General Meeting!!!  
Symposia...Posters...Exhibits...Workshops...Student  
Activities...Networking...Tours and More.*

Mark your calendars to attend ASM's Premier Annual Meeting and join us in Toronto for this unique showcase of the microbiological sciences.

**NEW!** ASM members can save up to 18% over the normal discounted registration rates during the registration period of November 10 - 30, 2006. Also, ASM members will have the opportunity to reserve housing at their primary hotel choice.

Registration and housing for non-ASM members will open December 1, 2006.

For more information, please view our website at <http://gm.asm.org>

## 2007

### Women's Career Development Grants for Postdoctoral Fellows

**...to encourage the careers of women with  
outstanding accomplishments and potential to  
carry out research in microbiology...**

Any woman scientist in the United States, holding a doctoral degree and performing postdoctoral work in the areas of microbiology represented by ASM scientific divisions, may apply. Up to two grants of \$1200 will be given annually.

#### Nomination requires:

- A candidate statement
- The candidate's CV
- A nominating and a seconding letter of support
- Candidates & nominators must be ASM members
- Nominations must be postmarked by **March 1<sup>st</sup>**

#### Mail nominations to:

ASM Membership Board Selection Committee,  
Women's Career Development Grants, American  
Society for Microbiology, 1752 N Street, N.W.,  
Washington, DC 20036-2804.

Additional information may be found on the ASM Website  
at <http://www.asm.org/Membership/index.asp?bid=37857>