

Invited Minireview—

Public Health Risk from Avian Influenza VirusesMichael L. Perdue^A and David E. Swayne^{BC}^ADepartment of Communicable Disease Surveillance and Response, World Health Organization, Global Influenza Programme, 1211 Geneva 27, Switzerland^BSoutheast Poultry Research Laboratory, Agricultural Research Service, USDA, Athens, GA 30605

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SUMMARY. Since 1997, avian influenza (AI) virus infections in poultry have taken on new significance, with increasing numbers of cases involving bird-to-human transmission and the resulting production of clinically severe and fatal human infections. Such human infections have been sporadic and are caused by H7N7 and H5N1 high-pathogenicity (HP) and H9N2 low-pathogenicity (LP) AI viruses in Europe and Asia. These infections have raised the level of concern by human health agencies for the potential reassortment of influenza virus genes and generation of the next human pandemic influenza A virus. The presence of endemic infections by H5N1 HPAI viruses in poultry in several Asian countries indicates that these viruses will continue to contaminate the environment and be an exposure risk with human transmission and infection. Furthermore, the reports of mammalian infections with H5N1 AI viruses and, in particular, mammal-to-mammal transmission in humans and tigers are unprecedented. However, the subsequent risk for generating a pandemic human strain is unknown. More international funding from both human and animal health agencies for diagnosis or detection and control of AI in Asia is needed. Additional funding for research is needed to understand why and how these AI viruses infect humans and what pandemic risks they pose.

RESUMEN. *Estudio recapitulativo por invitación*—Riesgos a la salud pública ocasionados por los virus de influenza aviar.

Desde 1997, las infecciones por el virus de influenza aviar en la población avícola han tomado un nuevo significado debido al incremento en el número de casos relacionados con la transmisión del virus a partir de aves a humanos y la subsiguiente presencia de infecciones clínicas severas y fatales en humanos, que han sido ocasionadas esporádicamente por virus de influenza aviar de alta patogenicidad H7N7 y H5N1, y de baja patogenicidad H9N2 en Europa y Asia. Estas infecciones han aumentado el nivel de preocupación por parte de agencias de salud humana debido al potencial de que ocurra un reordenamiento de los genes del virus de influenza y la generación del próximo virus pandémico humano de influenza tipo A. La presencia de infecciones endémicas ocasionadas por cepas de virus de influenza aviar H5N1 de alta patogenicidad en poblaciones avícolas de varios países de Asia indica que estos virus continuarán contaminando el medio ambiente y constituirán un riesgo de exposición con transmisión e infección en humanos. Los reportes de infecciones en mamíferos con virus de influenza aviar H5N1 y, en particular, la transmisión de mamífero a mamífero en humanos y tigres, no tienen precedente alguno. Sin embargo, se desconoce el riesgo a futuro de la generación de una cepa causante de una pandemia humana. Se requiere de mayores recursos económicos internacionales, tanto de agencias de salud humanas y animales, para el diagnóstico o detección y el control del virus de influenza aviar en Asia. Se requieren recursos adicionales destinados a la investigación para entender cómo estos virus de influenza aviar afectan a humanos y cuales son los riesgos que se presentan con respecto a una posible pandemia.

Key words: avian influenza, pandemic, public health, zoonosis

Abbreviations: AI = avian influenza; FAO = Food and Agricultural Organization of the United Nations; H1–16 = hemagglutinin subtypes 1 to 16; HP = high pathogenicity; LP = low pathogenicity; N1–9 = neuraminidase subtypes 1 to 9; OIE = World Organization for Animal Health (formerly Office International des Epizooties); WHO = World Health Organization of the United Nations

Influenza is primarily a respiratory disease caused by viruses in the family *Orthomyxoviridae* (reviewed in Swayne and Halvorson [102]). Infections of animals involve three genera; influenzaviruses A, B, and C. Type A viruses are the most common, and endemic infections have been reported in swine, horses, wild birds (especially the families Anseriformes [ducks, geese, and swans] and Charadriiformes [shorebirds]), domestic poultry, and humans. Sporadic infections have been reported in farmed mink, wild whales and seals, dogs, and captive populations of big cats (tigers and leopards) (48,102). The influenzavirus A (influenza A virus or type A influenza virus) genome is contained within eight gene segments that code for 10 different proteins. Influenza A viruses are further categorized, based on serologic reaction to the surface glycoproteins, into 16 hemagglutinin (H1–16) and 9 neuraminidase (N1–9) subtypes (35,102).

The gene pool for influenza A viruses is diverse and is maintained within wild aquatic bird populations. Over long periods of time, influenza A viruses or their genes are transferred between species either through *in toto* transfer of the whole virus with adaptation to the new host species or through reassortment of one or more of the genes with existing host-adapted influenza A virus genes to produce a hybrid virus. The latter occurrence is termed shift and is responsible for the emergence of hemagglutinin subtypes not typically identified in the animal or human populations. However, the mechanism through which pandemic viruses have emerged is unknown. The pig mixing vessel theory has been proposed as a mechanism because pigs are susceptible to both avian influenza (AI) and human influenza A viruses (85), but with the appearance of H5N1 high-pathogenicity (HP) AI virus infections in humans during 1997, direct reassortment between AI and human influenza A viruses could occur within the human without the need for an intermediate host. With the advent of surveillance tools and their use in the field for

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agricultural animals and humans plus molecular characterization of influenza A viruses from a variety of host species, the unfolding of the next avian–human reassortant influenza A virus may give us the opportunity to understand the mechanism of emergence of pandemic viruses and may aid in the development of intervention strategies to prevent the spread before it becomes a worldwide epidemic.

All AI viruses are influenza A viruses (102). Based on virulence for chickens using an intravenous pathogenicity test, AI viruses are classified into two pathotypes: i.e., low-pathogenicity (LP) and HP viruses. The HPAI viruses are defined as follows: 1) any AI virus that produces $\geq 75\%$ mortality or has an intravenous pathogenicity index of > 1.2 or 2) any H5 and H7 AI viruses that have a hemagglutinin proteolytic cleavage site compatible with a HPAI virus (64,102). All AI viruses that do not meet the criteria of HP are LPAI viruses. In chickens and related galliforme birds, the LPAI viruses cause primarily respiratory or reproductive diseases with low mortality rates, while HPAI viruses produce a severe, systemic disease with high morbidity and mortality. The vast majority of AI viruses are LP. AI viruses are maintained as LPAI viruses in the wild bird reservoir. Following transfer and circulation in domestic poultry, some H5 and H7 LPAI viruses have mutated to HPAI viruses, resulting in the 24 epizootics of HPAI documented since 1959 (Table 1).

Influenza A virus strains show host restriction or host adaptation, with the easiest and most sustained transmission between individuals occurring within the same species (95). Intraspecies transmission does occur, but it is infrequent, and transmission occurs most readily between host species that are closely related. Following transmission, the influenza A virus must go through adaptation to the new species before efficient and high-titer replication occurs. There is an association between efficient replication and expression of virulence (76).

HUMAN INFLUENZA

Impact of influenza infections in humans—the influenza burden. Twice each year the World Health Organization (WHO) convenes a meeting to make recommendations on which influenza strains should be included in “next year’s influenza vaccine.” This is a daunting task because the type A influenza viruses are continually evolving and adapting as the result of an inefficient RNA polymerase and perhaps in the face of immune pressure from “last year’s vaccine” or natural infections. Even with today’s vaccine technology and the knowledge that vaccines will prevent severe illness and death, only 300 million doses are produced and used worldwide in any given year, meaning that more than 95% of the world’s population remains immunologically naïve and at risk for infection and disease caused by newly emerging type A strains. So each year an estimated 250,000 to 500,000 people die worldwide as a result of influenza virus infections. When these yearly deaths from influenza epidemics are combined with those resulting from the massive pandemics that have occurred three times since 1900, influenza viruses become one of the deadliest human pathogens of the modern era. These data, of course, do not take into account the yearly economic losses from morbidity caused by the viruses. It can be estimated that in the United States, for example, the yearly economic burden caused by influenza deaths, infections, vaccinations, loss of productivity, and attendant health care costs is equal to 0.1%–0.5% of the gross domestic product (91).

The most significant impacts of influenza viruses on humans are those arising from the pandemic strains, and the deadliest of these was the so-called 1918 “Spanish influenza” H1N1 strain. It is often used as a modern-day example in infectious disease doomsday scenarios. Through a remarkable piece of molecular detective work, the full sequence of the 1918 pandemic influenza H1N1 strain has been

almost completely deciphered (79,105). We also now know much about the virulence contribution of several genes of this virus through a controversial regeneration process that has been carried out under careful containment procedures (10,50,108). Despite this work, however, the ultimate source of the 1918 pandemic strain remains a mystery. Most believe that the strain must have arisen initially from an avian source, but it clearly had to have spent some time adapting to mammals to have caused such a dramatic pandemic (78,79,104).

Although pandemic viruses tend to remain in the human population for several years as “seasonal influenza” following the initial wave of infections, the viruses can also simply disappear from the circulating virus populations, as happened when the 1968 H3N2 pandemic strain replaced the circulating H2N2 viruses that arose from the 1957 pandemic. This feature of influenza A viruses represents just one of the many ecological idiosyncrasies that make predictions tentative and burden assessments difficult. It is clear, however, that the influenza A strains have had profound effects on the human condition (9).

History of zoonotic transmission of AI. For the emergence of human pandemics in the 20th century, avian strains contributed to the process of zoonotic transmission through genetic reassortment and/or adaptation and fixation in the human population. The best evidence of involvement of AI viruses in human infections with influenza A strains comes from molecular characterization of viruses isolated during human pandemics (121). These pandemic strains clearly contained hemagglutinin genes that co-segregated or clustered phylogenetically with the avian-origin H2 and H3 strains circulating in nature. The 1957 pandemic virus had three genes (hemagglutinin, neuraminidase, and polymerase B1) from an AI virus, while the 1968 virus had two AI virus genes (hemagglutinin and polymerase B1) (47,77).

Since the discovery that fowl plague or HPAI was caused by influenza A virus (84), there have been several documented cases of human infections with AI viruses (Table 2). Sporadic individual or small clusters of limited human cases have been reported since 1959. In 1959, a H7N7 HPAI virus was isolated from the blood of a 46-yr-old man who developed hepatitis following his return to the United States from a 2-mo trip overseas through Asia, Africa, and Europe (26). He recovered, but no anti-H7-AI virus–neutralizing antibodies were detected. During the period ranging from 1978–79, self-limiting conjunctivitis was reported among workers who handled harbor seals experiencing an outbreak of H7N7 LPAI respiratory disease and death in the northeastern United States (114,116). During 1996, a 43-yr-old woman in England tending a collection of domesticated ducks of various breeds that mixed freely with wild ducks on a small lake developed self-limiting conjunctivitis from which a H7N7 LPAI virus was isolated (7,54). Between December 1998 and March 1999, a H9N2 LPAI virus was isolated from seven people, aged 1–70 yr, in mainland China and Hong Kong (73,95). Fever and respiratory disease were observed in the two Hong Kong patients, but signalment and symptoms were not reported for the five patients from mainland China. All patients recovered. In 2002, one H7N2 antibody-positive person was identified during the USA H7N2 LPAI outbreak in 2002, and in 2003, a patient with serious underlying medical conditions was admitted to a hospital in New York for respiratory symptoms and H7N2 LPAI virus was isolated. The patient recovered (18). Finally, symptoms of conjunctivitis, coryza, and headache were seen in two depopulation crew members in Canada from which an H7N3 HPAI virus was detected (111).

By contrast, the majority of the human AI infections and all the fatalities have involved Asian H5N1 (1996–2005) and the Netherlands H7N7 (2003) HPAI viruses (Table 2). The remaining sections will concentrate on the recent zoonotic transmission of HPAI viruses

in Asia and Europe and whether these transmissions pose significant threats of a new pandemic.

Importance/impact of 1997 H5N1 Hong Kong outbreak. In early 1997 a series of poultry outbreaks of HPAI occurred in Hong Kong SAR (89). In May a child fell ill with severe pneumonia, and the virus isolate recovered was typed as influenza A but was identified using standard reagents (H1, H2, and H3); a subtype was not determined. Screening the isolates for a variety of other subtypes and sequence analysis finally revealed the virus to be an H5N1 strain (24,94). Subsequent comparison of this strain with other similar human isolates and isolates from the poultry outbreaks revealed the chicken origin of the influenza A viruses that infected and killed humans (92). The direct transmission of a respiratory virus from birds to humans clearly signals a new level of concern. The Hong Kong outbreak was contained by rapid culling of infected poultry in the live market system with efficient environmental cleaning and disinfection and controlled restocking, but within 6 yr relatives of the 1997 H5N1 virus had returned with vengeance to nine Asian countries, causing large-scale outbreaks in poultry and new cases of human infection in three countries. Additionally, an H7N7 zoonotic outbreak in the Netherlands signaled a potential problem on the European continent. This dramatic and important increase in human infections by direct transmission of AI viruses initiated a wave of concern among the press and public health agency and officials worldwide, but the real risk of a pandemic is not clear. We need more genetic and biological data on these viruses to make better informed decisions in responding to the next potential pandemic.

HUMAN INFLUENZA SITUATION SINCE 1997

Hong Kong SAR, 1998–present. Although Hong Kong was considered the epicenter of the emerging H5N1 strains, it is likely that similar strains were circulating in mainland China prior to 1997 (115,122). Hong Kong's aggressive approach to solving the problem of HPAI exposure in humans (89) was facilitated somewhat by its unusual geographic circumstances. In any case, the authorities there carried out a successful campaign to rid the territory of H5N1, with brief reappearances in 2001 and 2002 (17). During this period, however, H9N2 LPAI virus infected eight people in three separate instances (73,95,119). This subtype is worrisome for three reasons. It appears to be widespread in other parts of Asia (22). Second, it has a receptor binding site that mimics in some ways the receptor binding site found in human strains (83). And third, it has been isolated directly from pigs and replicates in them (72). The Food and Agricultural Organization (FAO) and World Organization for Animal Health (OIE) have specifically recommended that countries increase surveillance for these H9 subtypes in Asia.

The February 2003 reappearance of the H5N1 virus in humans in Hong Kong resulted in two confirmed infections and a death, with three unconfirmed additional infections and another death in a Hong Kong family that visited mainland China (71). These cases were an apparent prelude to the wider outbreak in the region, but the source has not been identified, and Hong Kong poultry remain free of H5N1, largely because of an aggressive surveillance program in Hong Kong and successful vaccination approaches (32).

The Netherlands, 2003. The most widespread infection of humans by AI viruses in a single country was in the spring of 2003 in the Netherlands. A H7N7 HPAI outbreak began at the end of February 2003 in commercial poultry farms, and an investigation was carried out during the outbreak to determine whether transmission of virus from chickens to humans was occurring (51). Poultry work-

ers, farmers, and their families were screened for conjunctivitis or influenza-like illness. More than 400 people reported health complaints, with some 349 reporting conjunctivitis and 90 reporting influenza-like illnesses. One quarter of the conjunctivitis cases yielded the same H7N7 strains that were infecting poultry, and two of the influenza-like illnesses yielded virus. Alarming, six of the influenza-like illnesses were caused by H3N2 influenza A infections, meaning that these two viruses were cocirculating, although not from the same individual. One death attributed to the H7 virus was reported, and at least three contacts with documented cases yielded H7N7 virus, so it is very likely that human-to-human transmission occurred. So little is known about clinical progression and signalment of AI infections in humans; it is likely that many more conjunctivitis cases resulted from the virus. Serologic results have indicated a much wider-spread infection of humans during the outbreak (14).

Vietnam 2003, present. Since the massive endemic infection of H5N1 viruses in Southeast Asia began, Vietnam has been the hardest hit. Sixty-two of 64 provinces have been affected by the H5N1 in poultry, and 27 cities/provinces have suffered documented human infections, with 87 infections and 38 deaths. It is almost certain that the virus circulated in the country well before the large-scale outbreaks occurred, as H5N1 was recovered during a 2001 survey of live bird markets in Hanoi (63). It is also almost certain that many human cases have gone undocumented. The human cases in Vietnam, as is the case with those in Thailand, have appeared to follow a cyclical pattern (Table 3), peaking between December and March. It is difficult to ascertain whether this is the result of cooler weather or rather of increased contact and movement of poultry associated with the Tet Lunar New Year celebrations. The situation in Vietnam has provided an opportunity to follow the evolution of the H5N1 viruses in "real time," comparing animal strains with human strains as they transmit and cause disease. At the time of this writing, however, virus sharing has been inconsistent and disorganized between different countries and scientists. With the many WHO-, OIE-, and FAO-sponsored laboratories that are now capable of high-throughput sequence comparisons, it is unfortunate that this has created a dangerous knowledge vacuum regarding these viruses.

Thailand, 2004–present. The widespread poultry infections in Thailand have abated as of late spring 2005, and the country may soon announce that its poultry is HPAI free. A similar announcement also occurred in 2004, only to be followed almost immediately by another wave of poultry and human H5N1 HPAI infections. An FAO-sponsored study showed that the presence of H5N1 in the domestic duck population had a direct influence on the second wave of chicken infections (37). Control efforts have perhaps been more stringent and extensive in Thailand because of the importance of poultry exports to this country, as it is the world's fourth largest poultry exporter (33). The human infections in Thailand followed a similar pattern to that observed in Vietnam until 2005, when no new human cases were reported. Although it is tempting to speculate that the more extensive control efforts were effective in reducing the human deaths, it is difficult to make such statements, given the unknowns. Certainly domestic ducks are still harboring the virus in both countries, and the level of risk to humans is simply unknown.

The other troubling events in Thailand included a documented case of probable human-to-human transmission of the virus (18) as well as two separate documented series of infections by H5N1 in Felids. The first occurred at a zoo in Suphanburi, Thailand, and was shown to be due to the feeding of infected raw chickens to the tigers and leopards (49). The much more extensive infection occurred at a large cat farm in Chon Buri, south of Bangkok. In this outbreak, in addition to infection through the food source, probable cat-to-cat transmission was documented (106). Additionally, experimental evi-

Table 1. Twenty-four documented epizootics of HPAI since discovery of AI virus as cause of fowl plague in 1955 (modified from references 3,102,103).

Prototype AI virus	Subtype	Number affected with high mortality or that were depopulated ^A	Specific reference(s)
A/chicken/Scotland/59	H5N1	2 Flocks of chickens (<i>Gallus gallus domesticus</i>); total number of birds affected not reported	75 (Alexander, pers. comm.)
A/tern/South Africa/61	H5N3	1300 Common terns (<i>Sterna hirundo</i>)	13
A/turkey/England/63	H7N3	29,000 Breeder turkeys (<i>Meleagris gallopavo</i>)	117
A/turkey/Ontario/7732/66	H5N9	8,100 Breeder turkeys	55
A/chicken/Victoria/76	H7N7	25,000 Laying chickens; 17,000 broilers; and 16,000 ducks (<i>Anas platyrhynchos</i>)	6, 110
A/chicken/Germany/79	H7N7	Unknown (formerly East Germany)	3
A/turkey/England/199/79	H7N7	Three commercial farms of turkeys; total number of birds affected not reported	1, 5
A/chicken/Pennsylvania/1370/83	H5N2	17 Million birds in 452 flocks; most were chickens or turkeys, a few were chukar partridges (<i>Alectoris chukar</i>) and guinea fowl (<i>Numida meleagris</i>)	28, 29, 112
A/turkey/Ireland/1378/83	H5N8	800 Meat turkeys died on original farm; 8640 turkeys; 28,020 chickens; and 270,000 ducks were depopulated on original and two adjacent farms	2, 59
A/chicken/Victoria/85	H7N7	24,000 Broiler breeders; 27,000 laying chickens; 69,000 broilers; and 118,518 unspecified type of chickens	8, 25
A/turkey/England/50-92/91	H5N1	8000 Turkeys	4
A/chicken/Victoria/92	H7N3	12,700 Broiler breeders; 5700 ducks	86, 118
A/chicken/Queensland/95	H7N3	22,000 Laying chickens	118
A/chicken/Puebla/8623-607/94	H5N2	Chickens ^B	28, 113
A/Queretaro/14588-19/95			
A/chicken/Pakistan/447/95	H7N3	Three incursions: 1) 3.2 million broilers and broiler breeder chickens (northern part of country, 1994–95); 2) 300,000 chickens (central part of country, 2001); and 3) 2.52 million layers (Karachi, 2004) ^C	28, 61
A/chicken/Pakistan/1369-CR2/95			
A/goose/Guangdong/3/1996	H5N1	Between 100 and 200 million birds dead or culled, mostly chickens, but also ducks, geese, Japanese quail, and some wildbirds ^D	89, 90
A/chicken/Hong Kong/220/97			
A/chicken/New South Wales/1651/97	H7N4	128,000 Broiler breeders; 33,000 broilers; 261 emu (<i>Dromaius novaehollandiae</i>)	74
A/chicken/Italy/330/97	H5N2	2116 Chickens; 1501 turkeys; 731 guinea fowl; 2322 ducks; 204 quail (species unknown); 45 pigeons (<i>Columbia livia</i>); 45 geese (species unknown); and 1 pheasant (species unknown)	16
A/turkey/Italy/4580/99	H7N1	413 Farms: 8.1 million laying chickens; 2.7 million meat and breeder turkeys; 2.4 million broiler breeders and broilers; 247,000 guinea fowl; 260,000 quail, ducks, and pheasants; 1737 backyard poultry; and 387 ostriches	15
A/chicken/Chile/184240-1/2002	H7N3	Two farms, multiple houses; 617,800 broiler breeders, unspecified number of turkey breeders (two houses)	82
A/chicken/Netherlands//2003	H7N7	255 Infected flocks; 1381 commercial and 16,521 backyard/smallholder flocks depopulated. 30 million died or were depopulated, majority were chickens	31
A/chicken/Canada/AVFV2/04	H7N3	42 Commercial and 11 backyard flocks infected (1.2 million poultry), approximately 16 million commercial poultry were depopulated, most were chickens	44, 65
A/chicken/Texas/298313/04	H5N2	One noncommercial farm and two live poultry markets; 6600 chickens	56, 66
South Africa, strain name unavailable	H5N2	11 Ostrich farms with depopulation of 23,625 ostriches and 3550 other poultry (chickens, turkeys, geese, ducks, and pigeons)	69
North Korea, strain name unavailable	H7?	Three farms; 218,882 chickens culled; number dead not reported ^E	67

Table 2. Human cases of infection and fatalities with AI viruses (as of June 28, 2005).

Year	Country	Subtype/ pathotype	Cases	Fatalities	Reference(s)
1959	United States	H7N7 HPAI	1	0	26
1978–79	United States	H7N7 LPAI	?	0	114, 116
1996	England	H7N7 LPAI	1	0	7, 54
1997	Hong Kong	H5N1 HPAI	18	6	60
1999	China	H9N2 LPAI	5	0	41
1999, 2003	Hong Kong	H9N2 LPAI	3	0	73, 119
2002–03	United States	H7N2 LPAI	2	0	18, 19
2003	Hong Kong	H5N1 HPAI	5	2	71
2003	Netherlands	H7N7 HPAI	89	1	51
2004	Canada	H7N3 HPAI	2	0	111
2004	Thailand	H5N1 HPAI	17	12	120
2004–05	Vietnam	H5N1 HPAI	87	38	120
2005	Cambodia	H5N1 HPAI	4	4	120
Total			234	63	

dence clearly demonstrated the susceptibility of felids to these viruses and mammal-to-mammal transmission (53). The ability of these viruses to transmit from mammal to mammal has raised the concern level of public health officials.

Cambodia, 2004–present. In February of 2005, a Cambodian patient went to Vietnam for medical treatment, where she died and was confirmed to have been infected with HPAI H5N1 very similar to the circulating strains (120). Subsequently, three other deaths have occurred in Cambodia in the same southern province, and detection efforts are now being expanded with increased surveillance in humans and animals. As in Vietnam, the remoteness of many of the population centers make it difficult to know the extent of human and animal infections in the country, but based on population differences, it might be expected that fewer cases would be seen in Cambodia and Laos, compared to the more populous coastal neighbors of these countries.

The rest of Asia—the unknowns. China represents one of the great unknowns in this regional mix. There is clear evidence that as early as 1996, lethal H5N1 poultry infections were occurring (122). Serologic and virologic evidence also exists for infection of pigs in China, in addition to widespread infection of ducks (20) (Chen, pers. comm.). The remaining five affected Asian countries (Laos, Japan, Korea, Indonesia, and Malaysia) have reported poultry HPAI H5N1 infections but have not experienced clinical human AI virus infections, although sporadic sero-positive human samples for antibodies to H5 AI virus were recently identified in media reports from Japan (<http://www.flu.org.cn/news/200412256607.htm>) and Indonesia (<http://www.alertnet.org/thenews/newsdesk/JAK102004.htm>). In Indonesia, isolations of HPAI H5N1 viruses from pigs

Table 3. Confirmed AI cases and deaths in Southeast Asia (as of June 28, 2005; http://www.who.int/csr/disease/avian_influenza/country/cases_table_2005_06_28/en/index.html).

Period	Vietnam		Cambodia		Thailand		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
26 Dec. 2003–								
10 Mar. 2004	23	16	0	0	12	8	35	24
19 Jul. 2004–								
8 Oct. 2004	4	4	0	0	5	4	9	8
16 Dec. 2004–								
06 Apr. 2005	60	18	4	4	0	0	64	22
Total	87	38	4	4	17	12	108	54

have been reported, but it is not clear that the pigs were infected and rather may have simply been contaminated via close association with poultry waste. There is a critical need for expanded pig surveillance in Asia. A recent H5N1 HPAI infection that killed more than 519 birds from six different species in Western China has raised alarm, as this is the first report of H5N1 in the country since 2004 (68). This outbreak has dramatically demonstrated that ridding Asia of the virus is going to be a monumental undertaking.

ANIMAL MODELS TO STUDY HUMAN INFECTION AND PREDICT AI RISK TO HUMANS

Need for validated animal models. Currently there is no method for predicting which AI strains are capable of infecting and causing disease in humans. Having such a method, either by identifying unique molecular markers (see below) or with the aid of an animal model that would reflect human infections, would allow a full risk assessment to be done on the endemic situation in Asia, or would allow us to determine the level of containment/protection (e.g., use of personal protective equipment in the field) for depopulation crews in the case of epizootics strains. However, the problem is not confined to AI strains, as there is no ideal model available to assess the pandemic potential of the 1957 H2N2 derivatives. There are a few promising systems.

Various animal models have been used to study influenza A infections, including mice (*Mus musculus*), ferrets (*Mustela putorius furo*), various nonhuman primates, Syrian hamsters (*Mesocricetus auratus*), cotton rats (*Sigmodon hispidus*), chinchillas (*Chinchilla langera*), hedgehog (*Erinaceos europeaus*), Norway rat (*Rattus norvegicus*), guinea pigs (*Cavia porcellus*), and various birds (30,80). For the H5N1 and H7N3 studies, chicken, duck, geese, mouse, ferret, and primate studies hold the best promise in unraveling the complex biology of AI viruses.

^AMost outbreaks were controlled by “stamping out” or depopulation policies for infected and/or exposed populations of birds. Chickens, turkeys, and birds in the order Galliformes had clinical signs and mortality patterns consistent with HPAI, while ducks, geese, and other birds lacked or had low mortality rates or infrequent presence of clinical signs.

^B“Stamping-out” policy was not used for control. The AI outbreak had concurrent circulation of LP and HPAI virus strains. However, HPAI virus strains were present only from late 1994 to mid-1995. Estimates of number of birds infected with HPAI strains are unavailable, but 360 commercial chicken flocks were “depopulated” for AI in 1995 through controlled marketing.

^C“Stamping-out” policy was not used for control. Surveillance, quarantine, vaccination, and controlled marketing were used as the control strategy. The numbers affected are crude estimates from three separate time periods of clinical disease outbreaks, but the virus lineage is the same between the outbreaks.

^DThe H5 and N1 gene lineages have been maintained among the HPAI viruses from outbreaks in various Asian countries (1996–2005). The six internal gene segments have undergone reassortment. The initial H5N1 HPAI outbreaks were reported in China (1996), with three incursions in Hong Kong (1997, 2001, and 2002). This was followed by outbreaks in 2003–05 in South Korea, Vietnam, Japan, Indonesia, Thailand, Cambodia, Laos, China, and Malaysia. Initially chickens were the main species affected with disease and death, but in many of the outbreaks, domestic ducks have emerged to be a major species in maintenance and epidemiology of the viruses.

^EThis outbreak investigation was ongoing at the time of this article’s writing. The field epidemiology and signalment indicated an HPAI outbreak, but a virus was not available to an OIE reference laboratory for subtyping and pathogenicity determination.

Mouse models. The mouse model has been used extensively to study human infection with the Asian H5N1 viruses and has provided unique and valuable data for assessing the potential of the AI viruses to cause disease and death in humans. The mouse model has predictive value with regard to the potential virulence for humans once infected, but it is of questionable value in assessing the risk for human infection following exposure to AI viruses (i.e., infectivity) or the transmissibility of AI strain between individuals. Various strains of mice are available for use, and strain selection depends on the research question and study design. For example, BALBc is a genetically homogeneous, common, laboratory-strain mouse that is influenza A virus susceptible (Mx1-), while CAST/Ei is a wild-type, genetically heterogeneous laboratory mouse with resistance (Mx1+) to influenza A virus infection. In studying various H5 HPAI viruses, intranasal inoculation of three Hong Kong H5N1 HPAI viruses from 1997 into anesthetized BALBc mice produced severe disease and death, and such virulence did not occur with the H5 HPAI viruses for Mexico, the United Kingdom, and Italy (27). Some of the latter-group viruses produced mild respiratory infections but were not lethal or produced few deaths. Such data is in agreement with the field data, in which human lethal cases have occurred in Hong Kong with H5N1 HPAI viruses, and there has been a lack of human cases during previous H5 HPAI outbreaks. Even among the H5N1 Hong Kong AI viruses, the virulence for mice varied, with some being highly lethal, while others lacked lethality for the mouse model (42). With one virus strain, the lethality was associated with a mutation at position 627 of the PB2 gene and a highly cleavable hemagglutinin.

The genetics of the mouse model and the virus strain both contribute to infection and virulence outcomes. In another mouse modeling study, an H5N1 HPAI virus isolated from the first human case in Hong Kong (A/Hong Kong/156/97) was highly lethal (100%) in both BALBc and CAST/Ei mouse models, with virus replication occurring to high titers in trachea and lung (43). By contrast, H7N2 LPAI viruses replicated only in the lungs and trachea of BALBc mice, but caused no mortality, while the H7N2 viruses did not replicate in respiratory tracts of CAST/Ei mice. Field data corroborate these findings, since the Hong Kong H5N1 HPAI viruses infected and were lethal for human patients, while the H7N2 LPAI, despite numerous exposures, has only produced two cases of nonlethal infection: i.e., one with seroconversion and one with respiratory disease (Table 1). The latter case had a severe underlying medical condition that increased susceptibility to AI virus (18,19). However, we should not overinterpret mouse modeling data, which is only useful in predicting disease-producing potential for humans. First, wild mice are not a natural vector of AI viruses, as is evident by the inability to detect infection in wild-caught mice on farms having chickens infected with H7N2 LPAI during a 1996–97 outbreak (43). Second, the mouse model is not predictive for natural infectivity or transmissibility of influenza A viruses. To produce infection and lethality, the mouse requires intranasal inoculation while under anesthesia, especially with drainage of the inoculum from the nasal cavity into the lungs. Intranasal exposure without anesthesia or light sedation failed to produce infection in BALBc mice using a H5N1 HPAI virus (Swayne, unpubl. data).

Ferret. The ferret is perhaps the most promising model for studying human influenza infectivity, transmissibility, and virulence. Influenza virus infection in ferrets closely resembles the infection in humans with regard to clinical signs, pathogenesis, and immunity (58). Influenza A virus infections occur naturally in ferrets, and special precautions must be taken to avoid contact with infected humans during the rearing period to prevent human-to-ferret transmission and to prevent ferret-to-ferret transmission. With H5N1 HPAI

viruses, the ferret model has been more consistent in showing virulence than has been the mouse model. Ferrets intranasally infected with two human isolates from 1997 exhibited fever, weight loss, lethargy, and transient lymphopenia. In addition, the virus replicated in the upper and lower respiratory tracts and in multiple organs, including the brain (125). Using the ferret model, 2004 H5N1 HPAI viruses isolated from patients in Vietnam and Thailand were highly lethal and produced moderate to high titers of virus in internal organs, fever, and weight loss (40). By contrast, many of the 2004 avian-origin H5N1 HPAI viruses produced only a mild upper respiratory disease. Although the ferret has greater utility as a model for studying infectivity, transmissibility, and virulence, the presence of systemic virus and lesions has not been typically seen in human cases.

Primate. Various nonhuman primates have been used to study influenza vaccines and disease pathogenesis (80). Primate models are generally desirable because of the close relationship between primates and humans, their similar pulmonary anatomy and physiology, and their similar symptoms and lesion production. Recently, a cynomolgus monkey (*Macaca fascicularis*) model was used to study an H5N1 HPAI virus (A/Hong Kong/156/97) (52). In this model, the infection produced necrotizing bronchointerstitial pneumonia with alveolar damage, and virus localized only to the respiratory tract and tonsils, as was the case in primary influenza virus pneumonia of humans. The cynomolgus monkey and other primates comprise a suitable animal model for studying pathogenesis of human H5N1 virus infection.

Food and environmental safety considerations. Most of the recent H5N1 cases have been associated with direct exposure to live or dead infected poultry, but some cases suggest exposure only to raw poultry products (as reviewed in Swayne [99]). The demonstration of HPAI virus in meat, blood, and internal organs from HPAI-infected chickens and ducks raises the issue as to the role these vehicles could serve in transmission from birds to humans (101,109). Although cooking and pasteurization are effective at killing AI virus (98,100), the handling and consumption of raw or undercooked products could be a source of human exposure. This raises the question of the need for a model for enteric transmission in addition to the current respiratory models. It is not likely that these viruses will be food or waterborne agents in the classical sense of hepatitis A, polio, etc. They cannot withstand the pH of the gastric environment, but they could be a source of mucous membrane exposure. Other modes of entry must be employed since these viruses clearly show enteric involvement, and more animal research is needed to resolve the questions.

RELEVANT FEATURES OF THE ZONOTIC AI VIRUS STRAINS

Eurasian strains and zoonoses. A worthy goal for influenza researchers is determining why and how AI viruses are able to cross over from their avian hosts and infect humans. Certainly in all of the documented commercial poultry outbreaks prior to 1997 there were no indications of human infections, even though surveillance was carried out to identify human infections (11). Anecdotal evidence (lack of reports of human laboratory-acquired infections and limited mammalian susceptibility and human volunteer studies [12]) indicated that the likelihood of any infection with AI viruses was small. Indeed, even today, the chances of becoming infected with an avian respiratory virus of any kind are quite minimal.

So what has happened in the recent European and Asian strains that has allowed human infection? If a suitable animal model that reflects human infections can indeed be found, this question is much easier to answer by matching genetic changes with experimental

phenotypic changes. Looking at the geographic and phylogenetic data coming from analysis of hundreds of AI genes and strains over the last 15 yr, it is clear that at least two distinct phylogenetic clades of AI viruses exist: a North American and a Eurasian clade, as originally suggested (38,39). Almost all of the human infections are due to viruses within the latter clade. One clue of selective infectivity in mammals comes from comparison of capacity to cause disease in mice of various H5 HPAI viruses (27). In these studies it was shown that mouse lethality was correlated with transforming growth factor (TGF)- β levels in infected mice and that North American H5 strains did not showed lethality, infectivity, or suppression of TGF- β expression. The Asian H5N1 strains, on the other hand, were lethal in mice and showed no TGF- β expression. One European origin isolate showed intermediate pathogenicity and expression levels. Other characteristics of the human infectious or, more specifically, mammalian-infectious strains, include a slightly different polymerase gene with a specific amino acid difference at position 627 in mammalian-tropic strains, binding of host proteases by the viral neuraminidase to assist activation of the hemagglutinin, shortening of the neuraminidase, and a propensity to have characteristics promoting binding to human-type sialic acid receptors (42,88,93,124). Additional research is needed to better understand these contributions to pathobiology.

The occurrence of four documented cases of infection with H7N2 viruses in Canada and the United States may be a wake-up call to North America that the same potential for human infection exists here as in Europe and Asia. However, it is not clear whether these documentations represent improved diagnostics or a new susceptibility to avian viruses.

Transmissibility considerations. Human exposure to AI viruses occurs through contact with infected tissues, excretions, and secretions of infected poultry, principally feces and respiratory secretions, of which the latter contain the highest concentrations of virus (99). The AI virus can be transmitted through various media and mechanisms, including 1) inhalation of dust generated from infected poultry feces or respiratory secretions in the poultry farming environment; 2) inhalation of fine water droplets produced during slaughter and processing, especially at wet markets; 3) hand-to-mucous membrane (oral or nasal mucous membranes or the conjunctiva) transfer of infected feces or respiratory secretions from shoes, clothing, or environmental sources; 4) in a few cases, mucous membrane or inhalational exposure via mouth suction of clogged nasal passages of fighting cocks; and 5) theoretically, mucous membrane exposure through consumption of raw or undercooked blood, organs, or meat. However, there has been no confirmed epidemiologic evidence for the latter mechanism in human cases to date.

In general, the human infections have resulted from close direct contact with live or dead infected birds, principally chickens (99). In the 2003 the Netherlands H7N7 HPAI outbreak, people working closely with poultry during the depopulation (cullers, veterinarians, and poultry farmers) had the greatest risk for infection and clinical disease (51). For the 1997 Hong Kong H5N1 HPAI clinical cases, the primary risk factor was exposure to live poultry in the live (wet) market system 1 wk before the human illness, and infection was not associated with handling, cooking, or consuming poultry meat (60). In the early reported cases of H5N1 AI in Thailand and Vietnam, the primary risk was direct exposure to infected smallholder (village) poultry, and people involved with the culling operations or workers on commercial poultry farms were not among the cases (23,107).

Current research efforts and approaches to understanding transmission to humans. The current efforts to determine characteristics of AI viruses that allow them to infect mammals, including humans, have involved primarily 1) genomics

approaches to fully identify the molecular characteristics associated with those AI virus strains infecting humans; 2) the development of appropriate animal models (see above); and 3) use of recombination approaches to identify virulence-specific characteristics of individual genes. The most powerful approach is the reverse genetics systems that were recently developed (34,45,62) that allow creation of essentially any combination of influenza virus genes desired. It is probable that only certain combinations of gene segments will produce viable replicating organisms, but this procedure allows investigators to reshuffle specific genes and determine whether they influence virulence, replication, and transmission or host specificity; this procedure will be a critical tool in understanding avian-to-human transmission of AI viruses.

Role of host factors. A very interesting area of limited research involves understanding the host factors that may play a role in infections with AI viruses. Even though thousands of people must have been directly exposed to poultry infected with the Asian H5N1 viruses, relatively few have become infected, and a very small percentage have become clinically ill and died. Some data indicate that the early immunologic responses in humans to AI virus infections may play a role and that death due to the overreactions by the immune system (e.g., cytokine storms) may be occurring only in specific genetically susceptible individuals (21,57,87). Other possible genetic factors could include an increased susceptibility due to genetic variation in viral receptors or other cellular features that might allow more rapid spread within tissues. More human clinical studies are needed to answer these questions as well as to understand the clinical progression of the disease.

Other AI viruses. For the last 8 yr, the focus has been on the H5N1 HPAI viruses and the limited number of human cases. Despite the millions of human exposures, a reassortant virus containing human- and avian-origin influenza A virus genes has not emerged. The human influenza pandemics of the last century involved H1, H2, and H3 influenza A viruses, with contribution of the hemagglutinin genes from AI viruses. These three subtypes are all LP in avian species, and thus the high-mortality disease, as seen with the Asian H5N1 HPAI, did not occur as a clinical signalment before the last three human pandemics. Furthermore, there has been no evidence of a HPAI virus giving rise to a human pandemic virus. Could we be blinded by the severity of the HPAI virus in poultry into thinking it would be the next pandemic strain, when the actual risk may be LP AI of various subtypes? The H9N2 LP AI virus is endemic in domestic poultry in Asia, the Middle East, and Eastern Europe. In China alone, one survey of 221 farms found that 93% of the farms had chickens with antibodies to H9N2 AI (123). Other strains of LP AI have been reported from domestic poultry, including H6N2 and H7N2 LP AI viruses, and should be assessed for their risk. Which AI virus will contribute to the next pandemic strain is unknown, but vigilant surveillance is needed in veterinary and public health sectors to detect incursions of AI into animal and human populations. In addition, each AI virus strain should be assessed with regard to its risk for producing human infections, and mitigation strategies should be developed based on the risk to veterinary and public health. If each AI virus is treated as being a high risk for pandemic production, financial and personnel resources will be quickly exhausted in eradicating all AI viruses, and the public and scientific community will become disenchanted with the process.

POTENTIAL FOR GENERATION OF A PANDEMIC STRAIN

Concerns generated by the Southeast Asian outbreaks. Perhaps the most worrying aspect of the continued presence of the

H5N1 viruses in Asia is the unknowns that remain. There has been enormous recent press coverage of the potential for these viruses to become pandemic strains (see *Nature*, 435:May 26, 2005). Currently there appears to be at least some restriction on widespread transmission in humans of the H5N1 viruses because of three observed features. First, the AI virus strains that infect humans efficiently (owing to the more than 100 people who have been infected) appear to be limited to a restricted genotype (i.e., not all H5N1s in Asia are infecting people). Second, there have been few infections, even though there have been millions of exposures, so clearly there is either a dose-response restriction or a host restriction (see above). Third, clusters indicate that very close contact is required to infect other humans, and thus far this has occurred only with blood relatives (i.e., no husband-wife transmissions to date). Additionally, serologic surveys for infection of human contacts (e.g., health care workers and poultry cullers) in Vietnam and Thailand have thus far yielded uniformly negative results. It is probably dangerous to rely on the 'if it were going to happen it already would have' argument, but in fact most evidence indicates a difficult adaptation process for this virus into the human population.

A major worry is that at some point a seasonally recurring influenza H1N1 or H3N2 strain will infect an H5N1-infected human and a reassortant virus containing the correct combination of genes for efficient human-to-human transmission will emerge. With the low number of human cases of H5N1 in such a large population base, this would currently be statistically unlikely. Another issue is the unknown transmission efficiencies to other mammals and the likelihood of a coinfection of a mammal, such as a pig, with an avian and human influenza A virus strain. There is no question that it can happen; currently circulating H1N2 and H3N2 swine strains in both the United States and Asia contain influenza genes derived from viruses that once circulated in three different hosts: birds, humans, and pigs (46,70,81).

Controversial approaches. Many scientists feel it is important to embark on experiments to better evaluate the risk the H5N1 strains pose for a pandemic. Many of these proposed experiments are controversial because they involve using genetically engineered organisms to answer fundamental questions. Since we have the capability, should we do experimental studies to try and create potential pandemic viruses by reverse genetics to assess which avian viruses have a higher risk? Should we do *in vivo* reassortment experiments to determine which avian strains will actually reassort with which human strains? These are questions the answers to which may be crucial to our understanding of real pandemic risk.

Preventing infection of humans by poultry viruses and subsequent pandemic. What is the best approach? For AI in domestic poultry, there are three outcomes or goals of control programs: 1) to prevent, 2) to manage, or 3) to eradicate AI (97). To meet one of these three goals, control programs must be developed using universally recognized components. These components include management procedures to prevent introduction or escape of the pathogen at the farm level (biosecurity enhancements), detection of the pathogen or infections by the pathogen (diagnostics and surveillance), stamping-out acutely infected animals or controlled marketing of recovered animals, decreasing host susceptibility to the pathogen (vaccination or genetic selection of animal breeds/strains that are resistant), and education of people who produce poultry to better understand what AI is and to prevent its transmission or spread. The level of inclusion of these five components will determine whether the control program will prevent, manage, or eradicate AI.

There is no one single strategy that will uniformly prevent human infection with AI viruses (96); individual strategies must be tailored to fit within social, economic, and political conditions of each

country and must take into account the agricultural practices and AI status of the country. The absence of AI in domestic poultry will prevent exposure to humans and resulting infections, but the issues of H5N1 HPAI control in Asia are complex, and strategies must focus more on the high-risk smallholder farming sector than the lower-risk commercial operations. In developed countries, such as Japan and South Korea, immediate eradication of the H5N1 HPAI virus through detection and stamping-out was a successful strategy. This has been followed by increased efforts to prevent introduction of the H5N1 HPAI virus back into the country. In developing countries with numerous smallholder operations and limited financial-resources veterinary infrastructure, the H5N1 HPAI viruses have become endemic, and eradication by a similar program will not be successful in the immediate future. Management of the disease and reduction of infections is achievable through proper use of high-quality vaccines and concurrent use of diagnostics and surveillance along with eliminating infected flocks. Only after the disease can be managed and reduced in incidence will eradication be possible.

Working together—call for human and veterinary agencies to work more closely together. In comparing numbers, the current Asian outbreak is primarily an animal health problem. While it may be difficult to justify large-scale public health expenditures with relatively few and sporadic human infections, it is certainly easily justifiable to expect large-scale expenditures to solve the problem in animals with such potentially significant public health implications. A new Global Task Force (36) is really not needed and may simply provide another layer of cumbersome bureaucracy. What is needed is more donor funding for the existing animal global health agencies (e.g., OIE and FAO working with help from WHO) and the regional/country agencies that can effectively use funds to implement control of the animal diseases. Increased cooperation between the veterinary and human health agencies within countries is likewise a crucial component to control avian influenza at the animal-human interface and to manage the public health implications.

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