Genesis of pandemic influenza

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Abstract. During the last decade the number of reported outbreaks caused by highly pathogenic avian influenza (HPAI) in domestic poultry has drastically increased. At the same time, low pathogenic avian influenza (LPAI) strains, such as H9N2 in many parts of the Middle East and Asia and H6N2 in live bird markets in California, have become endemic. Each AI outbreak brings the concomitant possibility of poultry-to-human transmission. Indeed, human illness and death have resulted from such occasional transmissions with highly pathogenic avian H7N7 and H5N1 viruses while avian H9N2 viruses have been isolated from individuals with mild influenza. The transmission of avian

influenza directly from poultry to humans has brought a sense of urgency in terms of understanding the mechanisms that lead to interspecies transmission of influenza. Domestic poultry species have been previously overlooked as potential intermediate hosts in the generation of influenza viruses with the capacity to infect humans. In this review, we will discuss molecular and epidemiological aspects that have led to the recurrent emergence of avian influenza strains with pandemic potential, with a particular emphasis on the current Asian H5N1 viruses.

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Influenza A viruses are single stranded, negative-sense RNA viruses with a segmented genome (Fig. 1). They belong to the family *Orthomyxoviridae*. Influenza A is comprised of eight genes, encoding up to eleven viral proteins. The virus has an envelope with a host-derived lipid bilayer. It is covered with about 500 projecting glycoprotein spikes corresponding to the two major surface viral glycoproteins: the hemagglutinin (HA) and neuraminidase (NA). The HA is present as homotrimers and NA as homotetramers. HA and NA are the main antigenic determinants of the virus to which neutralizing antibodies are made. Influenza A viruses are classified by the antigenic properties of the HA and NA. Currently, 16 HA and 9 NA subtypes have been de-

scribed (Stallknecht and Shane, 1988; Fouchier et al., 2005). Within the envelope, a matrix protein (M1) and a nucleocapsid (NP) protein protect the viral RNA (vRNA) (Lamb, 1989). Approximately half of the total genome encodes the three viral polymerase proteins (segments 1, 2 and 3; PB2, PB1 and PA; Palese et al., 1977). Segment 5 encodes the NP protein. The three-polymerase subunits, the NP and the vRNA are associated in virions and infected cells in the form of viral ribonucleoprotein particles (vRNPs). Segments 4 and 6 encode the HA and NA genes, respectively. The two smallest segments (7 and 8) encode two proteins each with overlapping reading frames, which are generated by splicing of the co-linear mRNA molecules (Lamb and Lai, 1980, 1984; Lamb et al., 1981). In addition to M1, segment 7 encodes the M2 protein, which has ion channel activity and is embedded in the viral envelope. Segment 8 encodes NS1, a nonstructural protein that blocks the host's antiviral response, and NS2 (or NEP) that participates in the assembly of virus particles.

Virus entry occurs through endocytosis, facilitated by the HA attachment to sialic acid receptors present on glycoproteins or glycolipids on the cell surface (Helenius, 1992).

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Fig 1. Molecular structure of influenza A viruses. The virus contains a lipid bilayer derived from the host plasma membrane. Two surface glycoproteins (HA and NA) are the major antigenic determinants of the virus. The HA protein is responsible for binding sialic acid receptors. Human influenza viruses preferentially bind sialic acids in an alpha 2,6 conformation ($\alpha 2,6$ -gal), while those from avian species bind mostly to sialic acids in an alpha 2,3 conformation ($\alpha 2,3$ -gal). The virus also contains several copies of an ion channel proton pump (M2). Eight vRNA segments, associated to three polymerase subunits (PB1, PB2, and PA), and several copies of the nucleoprotein (NP) are located inside the virion protected by a protein mesh provided by the matrix protein (M1). In addition, the virus carries few copies of the virus encoded Nuclear Export Protein (NEP). In infected cells, the virus expresses NS1, which interferes with the antiviral state mounted by the cell. Some influenza strains express PB1F2, an ~80 amino acid peptide, derived from the second open reading frame of segment 2. PB1F2 has been shown to modulate apoptosis in certain cell types infected with influenza.

Once internalized, the acidic pH of the endosome triggers structural changes on the HA resulting in the fusion of the endosomal and viral membranes. Acidification of the endosomal lumen activates the ion channel activity of M2 (Pinto et al., 1992). M2 activation generates an inward current of protons that triggers the disassembly of M1 from the vRNPs. The latter are transported to the nucleus, the site of influenza virus transcription and replication (Martin and Helenius, 1991; Holsinger et al., 1994). The PB1, PB2, PA—referred to as P-proteins—and the NP protein (Huang et al., 1990) are the minimal set of viral proteins required for transcription and replication. Two different populations of positive sense RNAs are synthesized from vRNA templates: messenger RNAs (mRNAs) and complementary RNAs (cRNAs). Viral mRNAs are primed by 5' capped (m7GpppNm-containing) fragments derived from newly synthesized host-cell mRNA transcripts (Plotch et al., 1981; Ulmanen et al., 1983; Beaton and Krug, 1986; Krug et al., 1989). Viral mRNAs are polyadenylated by a stuttering mechanism involving the viral polymerase and a stretch of uridines, which are located 17–22 nucleotides before the 5' end of the vRNAs (Krug et al., 1989). Transcription of cRNAs occurs in the absence of primer or polyadenylation, represent full-length copies of vRNAs and are used for the synthesis of progeny vRNA molecules (Krug et al., 1989). Towards the end of the infec-

tion cycle, and once enough molecules of M1 and NEP have been produced, the newly synthesized vRNPs are exported out of the nucleus and assembled into full virus particles. The final assembly steps occur at the plasma membrane where the newly synthesized HA, NA, and M2 proteins are exposed (Helenius, 1992). New virus particles bud from the plasma membrane. The NA activity disrupts viral aggregates and thus viral particles are released.

In the 20th century humans experienced three influenza pandemics: the 1918 Spanish influenza (H1N1), the 1957 Asian influenza (H2N2) and the 1968 Hong Kong influenza (H3N2). Each strain led to global outbreaks and severe illnesses with high mortality, the most dramatic in 1918 in which 20–40 million people died worldwide (Hatta and Kawaoka, 2002). Genetic sequencing indicates that the HA and NA surface proteins of the 1918 influenza virus most likely emerged from an avian or swine reservoir (Taubenberger et al., 1997; Reid et al., 1999, 2000; Fouchier et al., 2004). The 1957 and 1968 pandemics arose through viral reassortment between human and avian influenza viruses (Scholtissek et al., 1978; Kawaoka et al., 1989). Thus avian influenza viruses (AI viruses) pose the threat of initiating pandemics in humans based on the fact that the human population is serologically naive towards most HA and NA subtypes.

Influenza A host range

All known subtypes of influenza A viruses circulate in wild aquatic birds which represent the origin of all viral genes for both avian and mammalian strains. Aquatic avian species can be infected by each of the 16 hemagglutinin (HA) and nine neuraminidase (NA) subtypes, in apparently any of the possible 144 subtype combinations (Webster et al., 1992; Fouchier et al., 2005). It is commonly accepted that migratory waterfowl including ducks, sea birds, or shorebirds, are responsible for introducing AI viruses into domestic poultry (Alexander, 2000). Some of these viruses can establish stable lineages in terrestrial birds (Order Galliformes) and a limited number of mammalian species including horses, pigs and dogs, among others. However for the purpose of this review we will focus on poultry species, please refer to reviews covering other hosts referenced here (Scholtissek, 1994, 1995, 1997; Webster, 1997; Crawford et al., 2005; Yoon et al., 2005; von Grotthuss and Rychlewski, 2006). Influenza A virus host specificity is in part mediated by the HA which binds to receptors containing glycans with terminal sialic acids. The majority of avian influenza viruses bind to receptors with sialic acids having an $\alpha 2,3$ linkage to the penultimate galactose (SA $\alpha 2,3$ -gal), while human viruses prefer receptors that are present with an $\alpha 2,6$ linkage (SA $\alpha 2,6$ -gal). The switch from $\alpha 2,3$ to $\alpha 2,6$ receptor specificity is thought to be critical in the adaptation of AI viruses to humans and appears to limit most AI viruses from directly crossing the species barrier (Beare and Webster, 1991). One way to overcome the preferential binding is through infection of animals that have both types of recep-

tors. Pigs carry both $\alpha 2,3$ and $\alpha 2,6$ receptors and are thought to act as intermediate hosts in which avian and human viruses can reassort and generate viruses with the ability to overcome the host barrier (Ito et al., 1998). Recent evidence suggests that other animals, particularly certain terrestrial poultry, may also provide an environment similar to pigs by displaying both SA $\alpha 2,3$ -gal and SA $\alpha 2,6$ -gal receptors (Gambaryan et al., 2002; Wan and Perez, 2006). Antigenic drift (small mutations on the surface proteins within a single subtype) drives a particular subtype to evade the host immune response and can provide the capacity to infect other animal species. If a host is simultaneously infected with two influenza subtypes, mixing of the genetic material from the two virus strains can take place, creating a completely new strain. Antigenic shift can then occur when a naïve population is infected by a novel strain carrying a completely different HA subtype (although antigenic shift could result in the emergence of a new NA subtype, the NA surface protein seems to play a less important role than the HA in terms of significance in disease outbreaks or pandemics). The combination of antigenic drifts and shifts could produce a strain that could cause excess morbidity and mortality in susceptible hosts such as those that have caused pandemics in humans.

Influenza A viruses have plenty of opportunities to cross the species barrier. The establishment of a stable lineage and the spread thereof is, however, a rare event (Hinshaw et al., 1980a, b; Webster et al., 1978). The likelihood of a virus becoming endemic in a new host (recipient species) depends on two major factors: at the macro scale level, the interaction between donor and recipient animal species, and at the molecular level, the intricate interactions between the host and virus components (Kuiken et al., 2006). Influenza A viruses appear well adapted to the wild bird reservoir in which infections are rarely accompanied by signs of disease (Perkins and Swayne, 2001). Due to complex interactions of the virus in various wild bird species, the mechanisms involved in the genesis of novel influenza strains and the epidemiological factors implicated in the emergence of pandemic outbreaks are poorly understood. Some HA and NA subtypes appear more prevalent in certain aquatic bird species than in others (Kawaoka et al., 1988). In addition, these viruses appear to undergo cycles of prevalence that may last a few months to several years.

Implications of domestic poultry in the spread of AI

Avian influenza viruses may infect several domestic poultry species; the progression and outcome of the infection, however, varies within each species. For example, a particular isolate may produce severe disease in turkeys but not in chickens or any other poultry. The most virulent forms of highly pathogenic avian influenza (HPAI) are characterized by a highly fatal systemic infection that spreads to most organ systems including the cardiovascular and nervous systems (Acland et al., 1984; Gross et al., 1986; Slemons et al., 1991; Perkins and Swayne, 2002a, b, 2003).

Morbidity and mortality can be as high as 100%, particularly in gallinaceous species. The incubation period is usually between 3 and 7 days depending on the virus isolate, age of the bird and bird species. Death may occur within 24 to 48 h after the onset of symptoms, but can be delayed for as long as one or two weeks. It is not the scope of this review to cover the clinical symptoms and histopathological aspects of the disease in different bird species, descriptions of which can be found in existing literature (Swayne and Suarez, 2000; Swayne and Halvorson, 2003). Avian influenza viruses from aquatic birds undergo significant selective pressure when adapting to chickens leading to definite changes in both the HA and NA genes (Matrosovich et al., 1999). The accumulation of basic amino acids at the cleavage site of the HA protein is a hallmark of the generation of avian influenza viruses with high pathogenic potential. Only AI viruses of the H5 and H7 subtypes have shown the potential to become highly pathogenic. An H5 or H7 virus can present itself as a low pathogenic virus but mutate without warning to become highly pathogenic, usually once they are introduced in domestic poultry, particularly chickens and turkeys (Kawaoka et al., 1984; Banks et al., 2001; Ito et al., 2001; Senne et al., 2006). Influenza subtypes other than H5 and H7 that can establish lineages in birds produce a mild disease, which can be exacerbated by secondary infections. For the HPAI viruses, the cleavability of the HA protein plays a critical role in the pathogenicity of AI viruses because it restricts tissue tropism (Kawaoka et al., 1987; Kawaoka and Webster, 1988; Walker and Kawaoka, 1993; Horimoto and Kawaoka, 1994, 1997; Rott et al., 1995; Steinhauer, 1999). HA is synthesized as a precursor, which requires post-translational cleavage by host proteases to create functional HA and produce infectious virus particles (Skehel and Wiley, 2000). All HPAI viruses identified to date differ from their low pathogenic avian influenza (LPAI) counterparts in the susceptibility of the HA to host proteases. HPAI are characterized by HAs that are highly susceptible to cleavage by numerous cellular proteases, which are ubiquitous in many cell compartments and organ systems. In contrast, the LPAI HA requires specific active extra-cellular proteases – such as trypsin – for cleavage and activation of infectivity. Trypsin-like proteases are restricted to the lumen of the respiratory and intestinal sites.

How do avian influenza viruses transfer from the wild bird reservoir into domestic poultry? One factor is agricultural and commercial practices that promote interspecies transmission and emergence of influenza viruses favoring the direct contact of natural hosts infected with influenza and non-natural hosts (Alexander, 1982). Live bird markets, backyard flocks, and free-range raised poultry are a few examples of such practices where natural and non-natural hosts of influenza can come in contact (Bulaga et al., 2003; Liu et al., 2003; Mullaney, 2003). These same conditions have also favored the transmission of avian influenza from poultry to humans.

The great variety of potentially susceptible domestic avian species increases the complexity and diversity of influenza strains in nature. Quail (*Coturnix coturnix*), for ex-

ample, have been shown to provide an environment where influenza viruses from the wild bird reservoir can increase their host range and infect other avian species (Makarova et al., 2003; Perez et al., 2003a, b). Quail appear more susceptible than chickens (White Leghorn) to avian influenza viruses from wild birds, typically harboring asymptomatic infections (Makarova et al., 2003; Perez et al., 2003a). Quail usually take longer than chickens to show signs of disease from HPAI, while shedding substantial amounts of virus, increasing the chances for the spread of the virus (Webster et al., 2002). Interestingly, the respiratory tract of quail possesses a pattern of expression of sialic acid receptors similar to the one observed in humans; i.e. ciliated cells that express abundant SAA2,6Gal receptors and mucin-producing cells that express SAA2,3Gal receptors (Wan and Perez, 2006). Other studies suggest that pheasants could possess similar characteristics to those observed in quail (Humberd et al., 2006). Furthermore, domestic bird species that are infected with LPAI often do not show signs of disease during the period when the virus is effectively transmitted, whether in live bird markets, back yard flocks, or large and small commercial poultry operations. From an epidemiological perspective, however, the prevention and control of avian influenza in aquatic domestic poultry is the most crucial (Hulse-Post et al., 2005). Several studies have pointed to free-grazing ducks as major determinants in the spread of H5N1 among domestic poultry in South East Asia (Gilbert et al., 2006). Domestic aquatic poultry has most likely been responsible for the spill over of H5N1 into wild aquatic birds and the consequent massive geographic spread (Martin et al., 2006). There is no precedent for the presence of an HPAI virus that can infect so many wild bird species as well as mammalian species (cats, humans, mice and ferrets) and with such lethal outcome. Previous reports of HPAI in wild birds have been characterized by either no mortality or limited spread. The current H5N1 strains have undergone cycles of increased virulence and mortality for wild birds and domestic ducks and other aquatic birds, while maintaining a typical HPAI phenotype for terrestrial poultry. The potentially devastating ecological consequences of such events remain to be seen.

The pandemic threat of H5N1

Before 1997, it was believed that molecular constraints restricted the host range of HPAI viruses to infection of avian species although limited cases of conjunctivitis were reported in humans (Campbell et al., 1970). Since the description of HPAI virus infection in 1878 as 'fowl plague', there has been little indication that the illness could be transmitted to humans, despite the multiple opportunities that have existed through the numerous outbreaks recorded in history. Since the 1990s, HPAI outbreaks have been reported frequently, caused by influenza viruses of subtype H5N1 in Southeast Asia (ongoing since 1997), H5N2 in Mexico (1994), Italy (1997) and Texas (2004), H7N1 in Italy (1999), H7N3 in Australia (1994), Pakistan (1994), Chile

(2002) and Canada (2003), H7N4 in Australia (1997), and H7N7 in The Netherlands (2003) (Alexander, 2000; Munster et al., 2005). Some of these outbreaks have been associated with transmission to humans causing respiratory infections, usually in people in direct contact with infected poultry. Previous serologic studies in the United States and Europe provided evidence of human infections with various HA subtypes of avian influenza from poultry, however these infections were generally subclinical (Shortridge, 1992). The exceptions were H7 HPAI virus infections in The Netherlands and British Columbia, Canada that occasionally caused conjunctivitis in humans, but also resulted in one fatality in The Netherlands (Fouchier et al., 2004; Tweed et al., 2004). Human infections have also occurred with LPAI H9N2 strains that are endemic in Asia and have acquired receptor-binding properties similar to those present in human influenza viruses. However, it is H5N1 viruses that have infected humans more often and with more devastating outcome. The H5N1 virus that was first shown to give rise to strains that can transmit to humans appears to have emerged in Southeast China. During 1996, an AI outbreak was observed in geese in Guangdong Province (Guo et al., 1998). Within a year, the goose virus reassorted, perhaps multiple times, with viruses circulating in other avian species (quail, chickens, teal). The new H5N1 virus was transported to Hong Kong, where it caused a major HPAI outbreak in chickens. The first bird-to-human transmission of this H5N1 virus was reported in a child that succumbed to the infection. Of the 18 human cases of H5N1 infection diagnosed at the time, six were fatal, prompting the rapid culling of approximately 1.5 million domestic birds in Hong Kong. This quick response likely succeeded in preventing additional human cases. Following the culling, the Hong Kong authorities adopted stringent prevention strategies that included the prohibition of aquatic birds in the live bird markets, monthly rest days in the poultry markets (during which lots were completely emptied and disinfected) and increased influenza surveillance in both wholesale and live bird markets. Unfortunately, similar strategies have not been implemented in other parts of Asia and, as a result, H5N1 viruses continue to circulate, particularly among geese and ducks, allowing genetic and antigenic evolution and increasing their geographic distribution and host range. Starting in 1999 in Hong Kong, avian species were monitored for virus through testing of blood and fecal samples and swabbing of cages. In May 2001, an avian H5N1 influenza virus was isolated from duck meat that had been imported to South Korea from China (Tumpey et al., 2002). Reports indicate that H5N1 circulated in Vietnam live bird markets during 2001 (Nguyen et al., 2005). From late 2002 through January 2003, mortalities of wild, exotic, and domestic birds due to HPAI were reported in Kowloon Park and other nature parks in Hong Kong (Ellis et al., 2004). In early February 2003, H5N1 viruses infected humans for the second time, resulting in two confirmed infections with one fatality (Peiris et al., 2004). Since late 2003, the world has experienced the largest outbreak of HPAI in birds in history. H5N1 outbreaks were reported in

Fig 2. World map of H5N1 avian cases since 2003 as reported to the WHO, OIE and FAO. Updated July, 2006. Reports of avian influenza outbreaks in domestic poultry (orange) and wild birds (yellow). Countries that reported outbreaks and have since been declared free of the disease by the OIE are highlighted with green triangles. Boxes represent number of human cases and deaths due to avian influenza for each of the corresponding countries.

Vietnam, Thailand, Indonesia, Cambodia, Laos, Malaysia, China, Korea, and Japan. Massive die off of bar headed geese, among other wild bird species, were reported in May 2005 from Qinghai Lake, one of China's most beautiful natural reserves. Subsequently, Russia (Siberia), Kazakhstan, and Mongolia reported outbreaks of H5 in wild and domestic birds in late 2005 (Food and Agriculture Organization reports, www.fao.org). Despite culling and vaccination strategies the virus has spread even further. H5N1 outbreaks have been reported in Eastern Europe, the Middle East, and Africa in late 2005 through 2006, including Romania, Turkey, Greece, Croatia, Ukraine, and recently in Nigeria, Bulgaria, and Italy. Human infections have also occurred in Turkey and Iraq. A complete list of countries reporting outbreaks in wild and domestic birds and humans is presented in Fig. 2 and Table 1. The overall mortality rate of confirmed cases is approximately 50%, and the possibility of a pandemic remains as long as this virus continues to circulate in birds. With the possible role wild birds play in the epidemiology of HPAI, large regions of Asia, Middle East, Europe and Africa have become at risk. The

HPAI H5N1 virus now poses a serious threat not only to Southeast Asia but also to the rest of the world.

Mechanisms of control: culling, vaccination, and the need for a global animal influenza surveillance network

Control of HPAI outbreaks have traditionally been resolved by the mass slaughtering of birds either infected or at high risk of becoming infected. This strategy must not be underestimated; it has been and will probably continue to be the most effective measure for the eradication of AI in infected flocks. However, when the viruses are widely spread in multiple domestic and wild avian species such as in the case of the Asian H5N1 epidemic, 'stamping out' alone is unlikely to be successful. Despite the approximately 150 million domestic poultry that have died or been culled in Asia to control the spread of the disease, the list of countries reporting outbreaks of H5N1 HPAI continues to increase. The estimated economic losses to the Asian poultry sector

are around \$10 billion, threatening the livelihood of millions of people (Perez et al., 2005). Thus other strategies to prevent and control the disease, such as vaccination, quarantine, and enhanced surveillance, have to be considered and used to complement stamping out policies.

Vaccination must be aimed primarily at reducing the presence of virus by reducing the number of susceptible animals or virus load. In Italy, vaccination of commercial poultry against H5 and H7 in high-risk areas combined with strict biosecurity measures and stamping out has resulted in the successful control of the spread of these viruses (Capua and Marangon, 2003; Capua et al., 2004; Marangon and Capua, 2006). However, no AI virus has been eradicated using vaccines alone. For example, continuous vaccination of chickens against a low-pathogenic H5N2 virus in Mexico over the last ten years has reduced disease signs and controlled the spread of the disease, however, the virus still circulates, particularly in the south of the country (Lee et al., 2004). The unnoticeable circulation of this virus may have contributed to its spread to neighboring countries including Guatemala and El Salvador. Influenza A vaccines in poultry include conventional inactivated oil-based whole AI virus, vectored virus, subunit protein and DNA vaccines. Currently, only inactivated whole AI virus vaccines and a fowl pox-vectored vaccine with an AI H5 HA gene insert are approved, prepared commercially, and used in various countries around the world (Suarez and Schultz-Cherry, 2000; Webster and Hulse, 2005). Current AI vaccines can provide a strong humoral response but typically do not produce a strong mucosal immune response and do not elicit adequate cellular immune response (Suarez and Schultz-Cherry, 2000; Wood et al., 2002; Capua and Marangon, 2004; Webster and Hulse, 2005). In regards to mass vaccination, current vaccines are associated with intensive labor requirements for parenteral administration, not to mention the cost of the vaccine, which is around 7 cents per dose.

Recent studies using live recombinant vectors have shown promising results with respect to efficacy as well as inexpensive methods for mass vaccination in poultry. In one of these studies it was shown that a live Newcastle disease virus (NDV) vector expressing the H5 gene from HPAI protected chickens from infection with NDV and HPAI (Veits et al., 2006). Likewise, an adenovirus-vector-based system expressing the H5 gene was shown to protect chickens against lethal challenge. An advantage of live vectors is that they are amenable to several vaccination strategies including aspersion and in ovo administration. A potential disadvantage of these systems is that prior exposure to the wild type agent limits the use of the vector. Studies are in progress to develop modified live attenuated avian influenza virus vaccines, which will have many of the benefits of live recombinant vectors and will not be affected by prior exposure to unrelated viruses or other agents.

Sporadic surveillance efforts have been key to the identification and characterization of potential epidemic and pandemic avian influenza strains. Since the late 1970's, live bird markets (LBMs) have been known to be a continuous source of influenza viruses (Shortridge, 1992). Neverthe-

Table 1. OIE Update on Avian Influenza in Animals (Type H5). Most recent official reports, 24 July 2006.

Afghanistan	Hong Kong	Pakistan
Albania	Hungary	Philippines
Austria	India	Poland
Azerbaijan	Indonesia	Romania
Bosnia and Herzegovina	Iraq	Russia
Bulgaria	Iran	Serbia and Montenegro
Burkina Faso	Israel	Slovakia
Cambodia	Italy	Slovenia
Cameroon	Japan	Spain
China	Jordan	Sudan
Cote d'Ivoire	Kazakhstan	Sweden
Croatia	Korea	Switzerland
Czech Republic	Laos	Thailand
Djibouti	Malaysia	Turkey
Egypt	Mongolia	Ukraine
France	Myanmar	United Kingdom
Georgia	Niger	Vietnam
Germany	Nigeria	Zimbabwe
Greece	Palestinian	
	Auton. Territories	

less, the current H5N1 situation warrants a better understanding of the ecology, epidemiology, genetics and evolution of influenza viruses. This information has to be analyzed in the context of the ecology of their natural hosts, and of environmental, agricultural, and social risk factors that lead to the emergence of novel influenza strains. Rapid and adequate responses to contain outbreaks require coordinated and experienced animal influenza surveillance structures capable of deploying and implementing high throughput analysis and characterization of influenza viruses obtained from either animal species or humans. Such structure is either deficient or lacking in many underdeveloped countries. Maintaining a global animal influenza surveillance structure will undoubtedly lead to quick implementations for the control and eradication of outbreaks and to the prevention of human infections. A transparent global network animal surveillance structure will also allow rapid communication among participating countries with respect to outbreaks and whether outbreaks in more than one location are caused by a single virus strain or multiple related or unrelated strains. Intensive surveillance plans are being coordinated by the international community through organizations like the Food and Agriculture Organization (FAO), World Health Organization (WHO) and World Organization for Animal Health (OIE).

Innate resistance to influenza in domestic birds

Since domestication, poultry has been considerably improved by natural and artificial selection. Modern selective breeding, particularly in chickens, has created significant progress in both egg and meat production (Romanov and Weigend, 2001; Burt, 2002). Current breeding strategies for commercial poultry focus on specialized production lines

bred from large populations of a few breeds with genetic uniformity for the desired traits (Notter, 1999). A major issue with this breeding strategy is the erosion of local breeds and the loss of valuable genetic traits (Romanov, 1996). As an example, the selective process for increased meat production in broilers has resulted in breeds with increased incidence of congenital disorders, reduced fertility and reduced resistance to disease. Likewise, layers have experienced an increase in osteoporosis associated with rise in egg production (Burt, 2005). The search for disease-resistant genotypes or traits could be beneficial to a comprehensive disease control program for poultry production.

Like in mammals, innate immunity in birds is considered to be mediated by type I interferons (IFN). IFNs are responsible for the induction of genes with antiviral activity prior to the development of adequate T-cell and B-cell responses during primary infection. The Mx protein family is one group of proteins that have antiviral activity and are induced in response to IFN. The majority of animal species analyzed to date have one to three Mx protein isoforms with different antiviral activities and intracellular localization (Lee and Vidal, 2002). Mx proteins are small GTPase enzymes that appear to interfere with RNA transcription during virus replication (Staeheli, 1990; Nakayama et al., 1991, 1992; Samuel, 1991; Horisberger, 1992; Pitossi et al., 1993). In mice and rats, a nuclear Mx isoform (Mx1) blocks replication of influenza virus (Staeheli et al., 1986; Meier et al., 1990), whereas the cytoplasmic form (Mx2) inhibits vesicular stomatitis virus (VSV) (Meier et al., 1990). The cytoplasmic human MxA protein shows antiviral activity in vitro against influenza, VSV, measles virus and Thogoto virus (Pavlovic et al., 1990; Haller, 1993; Schnorr et al., 1993; Horisberger, 1995). Birds, like mammals, produce Mx proteins, although their antiviral activity is less defined and remains controversial. The duck Mx, found in the nucleus and cytoplasm, does not appear to inhibit influenza replication, suggesting that the enhanced disease resistance to influenza in these birds is mediated by other unknown mechanisms (Bazzigher et al., 1993). Similarly, the chicken Mx protein, predominantly found in the cytoplasm, appears to have limited antiviral activity (Bernasconi et al., 1995). This protein is typically inactive in commercial breeds and attempts are being made to increase Mx expression or express the mouse Mx1 in chickens and evaluate its resistance to influenza and other viruses. Studies in transgenic pigs expressing the mouse Mx1 protein have yielded rather disappointing results in terms of enhanced anti-influenza activity (Muller et al., 1992).

AI viruses counteract the host's IFN response through the action of NS1, a small nuclear (~20 kDa), dimeric protein that is highly expressed in cells early in the infectious cycle and has dsRNA-binding activity (Hatada and Fukuda, 1992; Nemeroff et al., 1995). The NS1 interferes indirectly with IFN through several mechanisms. One of them includes the sequestration of dsRNA thus preventing downstream events required for the activation of IFN-pathway genes such as IRF3, NF κ B and AP1. The RNA-binding domain lies within the N-terminal 73 amino acids (Qian et al.,

1995). The C-terminal remaining portion of NS1 is called the effector domain and includes binding sites for poly (A)-binding protein II (PABII) and the 30 kDa subunit of cleavage and polyadenylation specificity factor (CPSF) (reviewed in Krug et al., 2003). In addition, NS1 has been reported to interact with several other host factors, including the eukaryotic translation initiation factor eIF4GI (Wolff et al., 1996, 1998; Falcon et al., 1999; Aragon et al., 2000; Burgui et al., 2003) and the protein kinase R (PKR) (Lu et al., 1995; Tan and Katze, 1998; Hatada et al., 1999). Activation of PKR in the presence of dsRNA (i.e. during virus infection) results in phosphorylation and inhibition of the translation initiation factor eIF2 α subunit and thus inhibition of protein synthesis. The blocking of PKR activity by NS1 permits proper translation of viral products and progression of the infectious cycle. Strategies could be envisioned to inhibit influenza replication by controlling the synthesis of NS1. The exploitation of the RNA silencing pathway through small interfering RNAs (siRNAs) is an attractive alternative that could be applied to induce influenza resistance in birds. RNA silencing has been shown effective in vivo against influenza (Tompkins et al., 2004). If siRNAs are developed against multiple viral genes the chances of resistant variants can be greatly reduced. However, with a moving target like avian influenza, genetic-resistant approaches have to be considered with moderate optimism.

Summary

The unprecedented outbreak of H5 HPAI in Asia that has spilled over areas of Europe and Africa has brought a sense of urgency in terms of understanding the mechanisms that lead to interspecies transmission of influenza. The realization that domestic poultry species can act as a reservoir for the emergence of novel strains of influenza with pandemic potential requires important changes in the way domestic poultry are produced and traded. However, these changes also require major international efforts in order to safeguard public health and protect poultry while respecting traditions and cultures. A sustainable global surveillance network with transparent and open communications should act as a platform to prevent and control future outbreaks. Ideally, countries would develop and implement policies that encourage and reward the reporting of suspected outbreaks. The development of resistant traits in poultry and improvement of vaccines will only help if biosecurity and education programs are properly implemented. By focusing our efforts at the source, i.e. poultry species, the emergence of influenza strains with pandemic potential can be greatly reduced.

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