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Review on Highly Pathogenic Avian Influenza H5N1 Viruses and Their Public Health Implications

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Abstract: The influenza virus is a complex and constantly changing virus. The physical structure of the influenza virus makes it particularly prone to small surface changes in antigens during replication which enables the virus to evade the host's immune system. This makes it possible for someone who has already been infected with influenza to become re-infected in subsequent years. There are two main types of influenza viruses of public health importance namely influenza A and influenza B. Influenza A viruses infect several different animals including pigs, horses, other mammals and aquatic birds as well as humans, whereas influenza B virus only infects humans. A minor change in one or both surface antigens of a virus may cause epidemics, because most people do not have enough antibody protection from past exposure to similar viruses. These small changes are known as antigenic drifts. A major change in one or both surface antigens (Antigenic shift) occurs only in type A influenza. An antigenic shift may cause a pandemic if the virus is easily transmitted from person to person. Because of the changes in the influenza virus, immunity to flu is short-lived and therefore large segments of the population are susceptible to influenza every year. New strains of influenza for which people have no immunity appear periodically, at irregular intervals, causing worldwide pandemics affecting vast number of people within short time-spans. From time to time, influenza A viruses in animals and birds jump species and infect humans. Recent H5N1 avian influenza epizootics associated with sporadic human fatalities have heightened concern that a new influenza pandemic, one at least as lethal as that of 1918, could be developing.

Key words: Antigenic drift · Antigenic shift · Antigenic variation · Avian influenza · Public health

INTRODUCTION

Avian influenza viruses are highly contagious, extremely variable viruses that are widespread in birds, particularly wild waterfowl and shorebirds. Most of these viruses, which are usually carried asymptomatically by wild birds, cause only mild disease in poultry. These viruses are called low pathogenicity avian influenza (LPAI) viruses. Others, the high pathogenicity avian influenza (HPAI) viruses, can kill up to 90-100% of a poultry flock. Epidemics of high pathogenicity avian influenza can spread rapidly, devastate the poultry industry and result in severe trade restrictions. Some avian influenza viruses can also infect mammals including humans. Although many human infections are limited to conjunctivitis or mild respiratory disease, some viral strains cause severe disease and death. Generally, avian influenza viruses do not spread efficiently in mammals and infections are limited to individual animals or small groups. However, some viruses can become adapted to a new species and cause epidemics or pandemics [1].

HPAI viruses have been eliminated from domesticated poultry in many nations including the U.S. and Canada; however, these viruses can be reintroduced from imported poultry, wild birds or pet birds. Raerly wild birds can carry HPAI viruses. More often, wild birds transmit LPAI viruses to poultry and these viruses then mutate to become HPAI viruses while they are circulating in poultry flocks. Although HPAI outbreaks can be devastating, the virus is successfully eradicated in most cases [2]. However, the world experienced an extensive avian influenza outbreak that has no immediate prospects for complete, worldwide eradication. In 2003, HPAI viruses of the H5N1 subtype appeared among poultry in

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several nations in Southeast Asia. Although at times this epidemic appeared to be under control, eradication was never complete. The outbreaks continued to smolder and spread and eventually Asian lineage H5N1 viruses reached other parts of Asia, Europe, Africa and the Middle East. The strains responsible for this epidemic appear to be unusually virulent. They have been found in many species of wild birds, which is unusual and numerous deaths have been reported in these species. As of January 2010, these viruses have also been responsible for approximately 470 human infections, generally as the result of close contact with poultry; about 60% of these cases were fatal. Asian lineage H5N1 viruses have caused disease in other mammals including housecats, several species of large felids, palm civets, raccoon dogs, stone martens, a dog and a mink. Some of these infections were fatal. In addition, these viruses have been detected in pigs and experimental infections have been established in a variety of species including foxes, ferrets, rodents and rabbits. There are fears that an Asian lineage H5N1 virus could eventually become adapted to humans, resulting in a severe human pandemic [3]. Therefore, the objectives of this article are:

- To review the importance of HPAI H5N1 viruses as cause of respiratory disease in human beings and other related risk factors.
- To highlight the state of HPAI virus outbreaks globally.
- To describe the viral characteristics that contribute to the development of new influenza viral strains.

Overview of Influenza a Viruses: Influenza viruses are enveloped, negative-stranded RNA viruses of *Orthomyxoviridae* family. They are classified as influenza A, B and C types. Of these types influenza A is the most pathogenic one. Influenza A virus can infect humans of all age groups and the infection in the population ranges from sporadic cases to large epidemics or pandemics. Influenza A strains also infect aquatic birds, pigs, horses and seals. Some of the strains isolated from animals are antigenically similar to strains circulating in the human [4].

Influenza viruses are significant cause of morbidity and mortality in human as well as avian and animal species worldwide. Antiviral drugs targeting influenza viral proteins, such as oseltamivir, zanamivir, amantadine and rimantadine, have been licensed for treatment of influenza. However, as influenza virus undergoes mutation very rapidly and can evolve drug resistance, the antiviral drugs can become ineffective during a flu outbreak [5].

Structure: Influenza A viruses are single-stranded RNA viruses with segmented genomes. They have a lipid envelope which is formed on exocytosis and have project of glycol proteins: hemagglutinin (HA) and neuraminidase (NA). HA mediates attachment and entry of the virus into the host cell, via binding with sialic acid receptors on the cell surface. NA facilitates the release of new viruses from the cell by cleaving the bond with sialic acid receptors. HA and to a lesser extent NA, are the main antigens against which host immune responses are mounted [6]. The virus also has two matrix proteins which link the viral envelope to its core. Matrix protein 1 (M1) interacts with the RNA genome and assists with the assembly of new virions. Matrix protein 2 (M2) acts as an ion-channel, which helps maintain the low pH necessary for viral unoating [7]. The genome has eight segments each of which exists in close association with nucleoprotein (NP) [8]. Each segment also has three polymerase proteins at one end (PA, PB-1 and PB-2) which are required for transcription. The virus makes two non-structural proteins, called NS1 and NS2 (Or nuclear export protein, NEP), whose roles are still being investigated [9].

Replication: The life cycle of influenza viruses begins when virions bind to sialic acid residues on the cell surface, allowing them to enter the host cell via receptor-mediated endocytosis [10].

Once inside the cell; the virus is trafficked through the endosomal network until acidification in late endosomes triggers the fusion of the viral envelope with the endosomal membrane. At the same time, vRNP undergo un-coating, i.e., dissociate from matrix protein (M1), which enables their release into the cytoplasm and import into the nucleus [11].

Unlike most other RNA viruses, the influenza virus replicates inside the nucleus of host cells. There, each vRNP acts as an independent functional unit that directs the synthesis of two positive-sense RNAs by using its vRNA as a template. First, viral messenger RNAs (mRNAs) are transcribed through a mechanism primed by capped RNA fragments that are cleaved from host pre-mRNAs. Since viral transcription terminates upstream of the vRNA 5 end, the resulting mRNAs are incomplete copies of the genome and thus cannot serve as the templates for replication. Hence, a second positive-strand, full length RNA is synthesized, the cRNA. In contrast to mRNA transcription, the replication of cRNAs is achieved via *de novo* initiation. Nascent cRNAs are encapsidated by newly synthesized NP and viral polymerases, leading to the formation of cRNPs. Similarly, *de novo*-initiated

synthesis from cRNPs generates vRNAs, which form progeny vRNPs. For incorporation into new virions, vRNPs have to leave the nucleus during the late phase of infection. Binding of M1 and of the nuclear export protein (NEP) thus mediates their export. Cytoplasmic vRNPs travel to the plasma membrane, where virus assembly and budding take place [12]. The regulation of viral RNA synthesis and especially the mechanism which controls whether vRNPs engage in the transcription of viral mRNAs or the replication of cRNAs is a matter of controversy. Early studies of virus-infected cells showed that replication requires an initial round of transcription and viral protein expression. It has since been well established that the NP protein in particular is an essential factor for cRNA accumulation [13]. Hence, NP would not induce a switch but merely act as a cofactor during cRNA encapsidation. This hypothesis is consistent with other observations showing that the relative rate of genome replication is independentof the abundance of NP and that the RNA-binding activity of NP is necessary for cRNA accumulation [14].

Influenza A Subtypes: Influenza type A viruses can infect people, birds, pigs, horses and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes and named on the basis of two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). For example, an“H7N2 virus designate an influenza A subtype that has an HA 7 protein and an NA 2 protein. Similarly an“H5N1” virus has an HA 5 protein and an NA 1 protein. There are 16 known HA subtypes and 9 known NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (H1N1, H1N2 and H3N2) are currently in general circulation among people. Other sub types are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses and H3N8 also has recently been shown to cause illness in dogs [15]. Only influenza A viruses infect birds and all known subtypes of influenza A viruses can infect birds. However, there are substantial genetic differences between the influenza A subtypes that typically infect birds and those that infect both people and birds [16].

Antigenic Variation: Antigenic variation refers to the mechanism by which an infectious organism such as a protozoan, bacterium or virus alters its surface proteins in order to evade a host immune response. It is related to phase variation. Immune evasion is particularly important for organisms that target long-lived hosts, repeatedly infect a single host and are easily transmittable. Antigenic variation not only enables immune evasion by the pathogen, but also allows the microbes to cause re-infection, as their antigens are no longer recognized by the host's immune system. The antigenic properties of influenza A viruses are determined by both hemagglutinin and neuraminidase. Specific host proteases cleave the single peptide HA into two subunits HA1 and HA2. The virus becomes highly virulent if the amino acids at the cleavage sites are lipophilic. Selection pressure in the environment selects for antigenic changes in the antigen determinants of HA, that includes places undergoing adaptive evolution and in antigenic locations undergoing substitutions, which ultimately results in changes in the antigenicity of the virus [17].

Acute viral infections can be rapidly cleared by the immune system of the host. Nevertheless, some viral infections like influenza and HIV recur. The recurrence occurs due to the production of virions that are resistant to the neutralizing antibodies that were able to effectively block the infection. These virions can infect survivors of the acute infection caused by the original virus. These viruses have a structural plasticity that enables them to tolerate changes in amino acids in their structural proteins while still retaining their infectivity. There is a lot of diversity in the ability of viruses to exhibit such plasticity. They can range from as little as 3 serotypes as in poliovirus to nearly 100 serotypes in rhinovirus [14].

Mechanism of Antigenic Variation: Most pathogens have large population sizes and short generation times. This is particularly true for viruses. The complete replication-cycle of a virus within a host cell often takes only a few hours and results in many thousands new viruses. Because the viral RNA-polymerase does not possess a proof-reading-function, faulty nucleotides are integrated during replication with a likelihood of 10-3 to 10-4, which results in high mutation rates. In fact, the error rate of the viral RNA-polymerase is 1000 times higher than the error rate of the human DNA polymerase. Thus, three characteristics contribute to the rapid evolution of these viruses: large populations, short generation times and high mutation rates. Every mutation, which enables its carrier to evade the host’s immune system, will be (Positively) selected, passed on to the next generation and distributed more widely. Influenza viruses evolve 1 million times faster than mammals. The ability of influenza viruses to evade the immune system and thus the persistence of the viruses in nature, is due to the

continuous evolution of new antigenic phenol types. This occurs through two genetic processes, called antigenic drift and antigenic shift [18].

Antigenic Drift: Antigenic drift is the gradual evolution of viruses due to the accumulation of random point mutations. Most point mutations are silent but if they occur within a section of RNA that codes for an antibody-binding site in the HA or NA protein, the binding of host antibodies may be affected. Immunity (Both naturally acquired or from vaccination) acts as a selection pressure that promotes the transmission of viruses with the ability to evade the immune system [19].

The RNA viruses acquire point mutations more frequently than DNA viruses, because they lack the repair function of DNA polymerases [20]. Viral RNA is replicated within the host cell. RNA-polymerase undertakes this task and has a relatively high error rate in comparison to DNA-polymerase. Also it does not have any proofreading-activity like other polymerases. Thus one nucleotide per viral generation changes on average. Numerous point mutations lead to (Minimal) changes in the genome, which can result in a change of the amino acid sequence. This continuous change of the genome is called 'genetic drift'. We speak of 'antigenic drift', in contrast, if the mutations affect the genes encoding antigens. The structure of these surface proteins can change minimally and therefore the immune protection of the host (Acquired through previous infections or immunization) will not be effective any more. As a consequence, the immune system cannot identify the new, minimally changed virus variants anymore and the lock-and key principle of the antigen-antibody-interaction is no longer fully functional. Variation through antigenic drift is the cause for annual influenza outbreaks [21].

All influenza viruses experience antigenic drift but the rate of drift is fastest in influenza A viruses. This is due to the greater selection pressure created by higher levels of partial immunity in humans to influenza A viruses, as well as the higher mutation rate of the virus [20]. Antigenic drift results in the occurrence of seasonal influenza epidemics. A high degree of antigenic drift is often associated with an early onset of a more severe influenza season [19].

Antigenic Shift: The recorded patterns of influenza A infection contain 2 phenomena; the first being the almost identical annual epidemics which occur in most countries and the second are the extensive pandemics which occur approximately every 10 - 12 years. It is apparent that pandemics are due to the appearance of new influenza A subtypes against which the population has no immunity [22]. Antigenic shift leads to the creation of viruses with new combinations of HAs and NAs. It occurs via the reassortment of RNA segments of two different virus subtypes (For example, avian and human subtypes) during co-infection of the same cell (Such as in a swine). The new virus has the potential to cause a pandemic, as immunity would not exist among humans [23].

Reassortment: Besides mutations, viruses with segmented genomes change genetically through 'genetic reassortment'. The latter term denotes the exchange of one or more genome segments between two related viruses which infect a host cell at the same time. During such a double infection the construction plans of both viruses become replicated in one host cell [24].

During the following assembling of the virus, mistakes in the combination of the segments can happen because the system cannot distinguish, which RNA-segments stem from which subtype of the virus. The reassortment of complete units of genetic material results in the formation of 'Reassortants' or 'Mosaic' viruses. At times, 'genetic reassortment' affects the exchange of genome segments encoding the viral surface proteins hemagglutinin and neuraminidase. Thereby, the virus achieves a new antigenic pattern. Thus, the process is called 'Antigenic shift'. While this is a common phenomenon, it is also the catalyst for pandemics among humans because pandemic viruses are often genetic reassortants of human and avian influenza-A virus-subtypes. Even though avian influenza-A subtypes usually cannot infect humans and even though poultry is usually not susceptible to human virus-subtypes, pigs play a very important role in the formation of new influenza viruses. They serve as a kind of 'melting pot' because they are susceptible to double infections with avian influenza viruses as well as human influenza viruses. In this way, new virus variants can be transmitted from pigs to humans. The risk of jumping the species barrier between birds and humans is particularly high for the lethal avian flu virus H5N1, even though it caused only few infections of humans until now [23].

Benefits of Antigenic Variation: Infectious disease remains a major cause of morbidity and mortality. Consequently, great research effort has been devoted to organisms and to host immune responses that fight these

organisms. This has led to rapid progress in understanding the biology of viruses, including the molecular details about how viruses invade hosts and escape host immune defenses [25].

Extend Length of Infection: Some viruses, such as HIV, escape immune attack by mutating their dominant epitopes. Mutational changes to new, successful epitopes may be rare in each replication of the virus. But the very large population size of viruses' with in a host means that mutations, rare in each replication, often occur at least once in the host in each generation [26].

Infect Hosts with Prior Exposure: Host immune memory recognizes and mounts a rapid response against previously encountered antigens. Antigenic variants that differ from a host's previous infections escape that host's memory response [27]. The distribution of immune memory profiles between hosts determines the success of each variant. Each variant may occasionally spread epidemically through the host population. This leaves a large fraction of the hosts resistant upon recovery, driving that particular variant down in frequency because it has few hosts it can infect [28].

Vary Attachment Characters: Surface antigens often play a role in attachment and entry into host cells or attachment to particular types of host tissue. Varying these attachment characters allows attack of different cell types or adhesion to various tissues. Such variability can provide the variant with additional resources or protection from host defenses [29].

Evolution of H5N1 Viruses, 1997–2004: In 1996, an H5N1 virus was isolated from geese during an outbreak in Guangdong Province in China (Influenza A/Goose/Guangdong/1/96 (A/G/Gd/96). This virus proved to be the donor of the hemagglutinin (HA) gene of the reassortant H5N1 viruses causing the outbreak among poultry and humans in Hong Kong in 1997. The internal genes of the Hong Kong H5N1 viruses were closely related to those of an H9N2 virus isolated from quail. The origin of the Neuraminidase (NA) gene remains unclear, but was notable for a 19-amino acid deletion in the stalk region. Such deletions may be associated with adaptation of influenza viruses to land-based poultry [3]. The HA gene contained multibasic sequences at the cleavage site, in accordance with its classification as a highly pathogenic strain [30].

After the eradication of the 1997 Hong Kong strain, the goose precursor viruses continued to circulate in geese in southeastern China [31]. Through reassortment between this virus and other avian viruses, multiple antigenically similar genotypes, that were highly pathogenic in chickens but not in ducks, emerged and again were eradicated in Hong Kong in 2001 and 2002 [3]. Then, in late 2002, H5N1 strains isolated from wild migratory birds and resident waterfowl in two Hong Kong parks showed marked antigenic drift and exhibited high pathogenicity in ducks. The latter property is rarely found in nature and had not been observed in strains isolated during previous years. An antigenically and molecularly similar virus caused the two confirmed human infections in early 2003 in a family from Hong Kong [3]. The H5N1 influenza viruses isolated from healthy ducks in southern China between 1999 and 2002 were all antigenically similar to the precursor influenza A/G/Gd/96 virus [32].

It is thought that these ducks played a central role in the generation of the virus responsible for the outbreaks in Southeast Asia since 2003. Detailed genetic analyses of H5N1 strains isolated during the period 2000–2004 from poultry and humans in China, Hong Kong, Indonesia, Thailand and Viet Nam, demonstrated that a series of genetic reassortment events, all traceable to the A/G/Gd/96-precursor virus, ultimately gave rise to a dominant H5N1 genotype (Genotype Z) in chickens and ducks. This genotype is implicated in the human cases in Hong Kong in 2003 and the outbreaks among poultry and humans since 2004 [33].

The evolution of H5N1 viruses in recent years has been associated with increasing virulence and an expanding host range, which beside terrestrial poultry and wild birds, also includes mammals. While all H5N1 viruses isolated from ducks in China between 1999 and 2002 were highly pathogenic in chickens, an increasing level of pathogenicity was observed in mice with the progression of time: virus isolated in 1999 and 2000 were less pathogenic than those isolated in 2001 and 2002. It has been suggested that the increasing ability to replicate in mammals has resulted from transmission between ducks and pigs. The expanding host range is also illustrated by successful experimental infection of domestic cats and natural infections of tigers and leopards with recent H5N1 strains [32].

Public Health Implications of H5n1 Avian Iinfluenza Viruses: Due to the recent cases of human infection caused by AI viruses and to concern about the generation of a new pandemic virus originating from the

H5N1 virus, AI infections are now considered a significant threat for public health. Although it has been known for some time that the human pandemic viruses of 1957 and 1968 appeared to arise by reassortment between viruses present in the human population and AI viruses [3] because of the apparent "barriers" to human influenza viruses infecting birds and AI viruses infecting humans, it was suggested that pigs, which both human and avian viruses are known to infect readily, acted as "mixing vessels." The scientific basis for this was that pig epithelial cells contain both SA-α2, 3-Galterminated saccharides and SA-α2, 6-Gal-terminated saccharides and this allows the replication of both avian and human influenza viruses. Reassortment between human and avian influenza viruses could therefore take place in pigs, with the emergence of viruses with the necessary gene(s) from the virus of human origin to allow replication and spread in the human population, but with a different haemagglutinin surface glycoprotein, so that the human population could be regarded as immunologically naive. At least two AI subtypes, H5N1 and H9N2, both of which have zoonotic implications, are currently apparently endemic in vast areas of the world. It is impossible to predict whether either of them will represent the progenitor of the next human pandemic virus. Certainly, both of them are causing losses to the poultry industry and H5N1 is also responsible for the loss of human lives and the reduction of the livelihood of rural establishments [19].

Human H5N1 infections and Related Risk Factors in Different Parts of the World

Asia

Hong Kong: The H5N1 virus first crossed the animal and human species barrier in 1997 in Hong Kong in a 3 year old boy and subsequently infected 17 others. A case-control study of 15 of these confirmed H5N1 cases and 41 controls matched on sex and age found that exposure to live poultry at markets in the week before illness was associated with a 4-fold increased risk in infection with H5N1; but did not find consumption of cooked or undercooked poultry at home or at a restaurant as risk factors for infection [3].

Vietnam: There have been 106 cases and 52 deaths due to H5N1 infection in Vietnam since 2003.The majority of these cases were detected in 2004 and 2005 and incidence has declined possibly due to reduced exposure resulting from control of HPAI in poultry through mass vaccination of domestic poultry populations [22].

A case-control study from Viet Nam found increased risk for human infection with H5N1 from preparing/cooking unhealthy poultry, having sick or dead poultry in the household, presence of sick/dead poultry in the neighborhood and no indoor water source in the household. This study did not find any other risk factors for infection including other animals in the household or neighborhood (Pigs, dogs, cats, buffalo and cows), household members working with commercial poultry, helped prepare or cook sick or dead poultry, prepared and cooked healthy poultry, or bought freshly killed poultry for household consumption [20].

Thailand: There have been 25 cases and 17 deaths due to H5N1 infection in Thailand since 2005. All of these cases occurred in 2004-2006, none have been reported since 2006. A case-control study from Thailand evaluated risk factors for H5N1 infection in 16 confirmed patients as compared to 64 controls matched on village and age. Cases were more likely to have touched a dead bird that died unexpectedly; had poultry that died unexpectedly around their house; plucked feathers from poultry; stored products of sick or dead poultry in their house; and directly touched sick poultry. Risk factors for infection also included living ≤1 meter from sick or dead poultry [22].

Africa: Africa recorded its first outbreak of H5N1 when Nigeria first reported outbreaks of HPAI in domestic poultry on February 8, 2006. Seven other African countries (Egypt, Niger, Cameroon, Burkina Faso, Sudan, Côte d'Ivoire and Djibouti) subsequently reported H5N1 infection and disease in their poultry flocks. In Burkina Faso, Cameroon, Djibouti, Niger and Côte d'Ivoire the disease has remained relatively localized, with the spread and impact of the disease being greatest in Egypt, Nigeria and Sudan. As of November 13, 2006, the total number of human infections reported was 258, of which 153 people died, representing a case-fatality rate of 59.3%. Egypt has recorded 15 human cases with 7 fatalities. In Djibouti, a young girl fell ill after she became infected with the H5N1 virus. Given the deficiencies in surveillance systems in many African countries, it is possible that the apparent localization of reported cases might not reflect the actual distribution of the virus and further spread of HPAI in Africa, by way of migratory birds or through trade, is thus very likely [6].

Pandemic Preparedness and Future Directives: The increasing frequency of outbreaks with highly pathogenic avian influenza viruses among poultry and direct

transmission of these viruses to humans, culminating the extensive H5N1 outbreak in Southeast Asia, has ignited grave concerns about an imminent influenza pandemic. Indeed, two of three prerequisites for a human pandemic have been met in the Southeast Asian H5N1 outbreak: the emergence of an antigenically novel strain to which the population has no immunity and the transmission of this strain to humans in whom it can cause severe disease [17]. To date, there is fortunately no evidence of efficient spread of H5N1 virus between humans, but continued circulation of this strain, which now has reached levels of endemicity among poultry in several Southeast Asian countries, increases the opportunity to adapt to humans through mutation or genetic reassortment in humans or intermediate mammalian hosts. As suggested by the "Spanish flu" pandemic of 1918, extremely high transmissability is not prerequisite for a severe pandemic and as shown by the Severe Acute Respiratory Syndrome (SARS) virus epidemic in 2003, viruses can rapidly spread across the globe in the current age of intense global travel. As a consequence of all this, pandemic preparedness has become an important issue worldwide. Pandemic plans, which include stock-piling of antivirals and candidate vaccines, are being developed by an increasing number of countries worldwide and alternative methods for rapid vaccine production and potential methods enabling dose reduction of vaccines are increasingly propagated [20].

Notwithstanding the importance of these efforts to prepare for a possible H5N1 pandemic, more structural and longer term global efforts are needed to allow for early recognition of novel influenza viruses or other emerging pathogens infecting humans in the future. In 2002, a WHO Global Agenda for Influenza Surveillance and Control has been adopted, of which the main objectives are to strengthen surveillance, improve knowledge of the disease burden, increase vaccine use and accelerate pandemic preparedness [34].

It is essential that these objectives are increasingly focused on the Southeast Asian region. Many Southeast Asian countries currently lack the expertise, financial means and infrastructure for human and animal diagnostics and surveillance. Global investments to improve public health care infrastructures and laboratory facilities and to transfer clinical, epidemiological and technical knowledge to these countries are much needed. The window of opportunity in the era of global travel is narrow [35]. Local capacity and less dependence on foreign laboratories and expertise, will allow for earlier recognition and quicker responses to epidemics. In addition, local availability of clinical, scientific and laboratory capacity facilitates and expedites clinical, virological and epidemiological analyses needed to optimize outbreak control, infection control and clinical management. It also guarantees the timely availability of virus strains for monitoring virus evolution and planning of vaccines by reference laboratories. Such global investments to enhance local infrastructure and expertise will increase the chances of success of containing an influenza pandemic at the source by antiviral prophylaxis and other preventive measures suggested by recent mathematical modeling studies [36].

CONCLUSION AND RECOMMENDATIONS

The rationale for particular concern about an H5N1 pandemic is not its inevitability but it is possible severe impact on human health. Such a pandemic, especially if it arises by direct adaptation rather than genetic reassortment with a preexisting human virus, could well be unusually virulent in humans. Thus, an H5N1 pandemic is an event of low probability but one of high human health impact. What is certain, however, is that the H5N1 panzootic already impacts human health via its economic and consequent nutritional impacts on rural societies and by occasional zoonotic transmission, leading to severe human disease with its attendant social impact. Given the increasing geographical spread and the endemicity of H5N1 viruses in poultry across the world and its possible (Yet-to-be-proven) foothold within wild bird populations, H5N1 is likely to remain a serious threat to human health for quite some time to come. Taking these facts into consideration, the following recommendations are forwarded:

- Collaboration between human and animal health sectors (One health approach) is essential to understanding the transmission dynamics of H5N1viruses among wild birds, domestic birds and humans
- There is a need for continued high levels of awareness and surveillance to monitor emerging influenza viruses.
- Control programs of zoonotic avian influenza should aim the control of the virus in birds including wild birds which are the supposed source of the infection.

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