Highly Pathogenic Avian Influenza (HPAI) H5N1 Virus in Asia: Evolution and Vaccination

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Abstract

HPAI (H5N1) is still an important emerging disease posing threat on both human and animal health. The causative agent continues to evolve rapidly within various poultry populations and may cause unpredictable outcome. Evolution of the virus will continue until it reaches equilibrium. At present, the HPAI (H5N1) viruses still possess avian virus characteristics. However, if the viruses gain opportunities to infect and evolve in human, it may accelerate adaptation of the avian viruses to be more human preference and thus acquires ability to infect and transmit efficiently among human population. This review aims to elucidate crucial issues regarding to role of vaccination in virus evolution including influence of immune response after vaccination and adaptation of virus to cross species. Additionally, role of vaccination in HPAI (H5N1) control and drawback of vaccine usage are discussed.

Keywords: Avian influenza virus, H5N1, evolution, interspecies transmission, vaccination, disease control.

Introduction

HPAI (H5N1) initially reported in poultry farms in Hong Kong in 1997 and was eradicated by culling of all chicken (Li et al., 2004; Lipatov et al., 2004). Thereafter, the disease was reemerged in Hong Kong prior to widespread throughout Asian countries including China, Korea, Japan, Vietnam, Thailand, Lao PDR, Cambodia and Malaysia in 2003. The virus killed large numbers of domestic poultry and infected over 40 people with more than 50% cases fatality in 2004 (World Health Organization, 2007). In mid 2005, the HPAI (H5N1) infected and killed thousands of migratory birds in Qinghai Lake of China (Chen et al., 2006b) before it was rapidly spread to East Asia, Eurasia, Europe and Africa (Salzberg et al., 2007; World Organization for Animal Health, 2007). Recently, the outbreaks have extended to 67 countries over the world (World Organization for Animal Health, 2008a) and caused 387 hospitalized with 245 fatal cases in human from 15 countries (World Health Organization, 2008a). This situation demonstrates that the HPAI (H5N1) virus can cross barriers of the interspecies transmission and poses pandemic threat in human. In addition, HPAI (H5N1) is a highly virulent and contagious disease for poultry that requires combination of various strategies including vaccination to control spreading of the disease in poultry population and to decrease viral load in the environment (Food and Agriculture Organization and World Organization for Animal Health in collaboration with World Health Organization, 2005).

Evolution of HPAI (H5N1) in Asia

Avian influenza virus is classified in genus *Influenza A virus* of family *Orthomyxoviridae*. The genomic RNAs are negative sense and comprise of eight segments: polymerase basic (PB) 1, PB2, polymerase acid (PA), HA, Nucleoprotein (NP), Neuraminidase (NA), Matrix (M) and Nonstructural (NS) genes. The HPAI (H5N1) viruses isolated from poultry and human in Hong Kong in 1997 may have acquired HA and NA from geese influenza viruses in Guangdong of China (A/Gs/Gd1/96) and the remaining six genes from different sources of viruses by multiple reassortments (Li et al., 2004; Lipatov et al., 2004). Despite eradicated from Hong Kong, the Gs/Gd/96 virus still circulated in the geese population residing in southern China. It continued to exchange their genes with other avian influenza viruses perpetuating in the common habitat and thus resulted in various genotypes such as V, W, X, X2, X3, Z and Z+ (Li et al., 2004). Before 2002, the antigenic characteristics of HA from different genotypes of avian influenza viruses subtype H5N1 were relatively stable. However, the virus isolated in 2002 showed alterations

within the HA gene indicating antigenic drift. The viruses also produced clinical signs in wild ducks and aquatic birds. Thereafter, the HPAI (H5N1) reemerged once again in 2003. The Hong Kong 2003 virus is genetically and antigenically similar to the viruses isolated in 2002.

In 2004, the HPAI (H5N1) was spread and caused serious epizootic outbreaks in Asia including China, Japan, Korea, Vietnam, Cambodia, Laos and Thailand. The Southeast Asia viruses are classified as genotype Z (Li et al., 2004). However, the viruses from Japan and Korea are genetically different and classified as genotype V (Mase et al., 2005). The HA and NA genes of these viruses are closely related to those of the Gs/Gd/96 ancestor virus. The viruses become endemics in several countries and continue to evolve separately in different geographical areas. Analysis of HA genes of the Asian HPAI (H5N1) viruses isolated from 2003 to 2005 revealed six clusters of the viruses corresponding to each geographical areas (Amonsin et al., 2006; The World Health Organization Global Influenza Program Surveillance Network., 2005; World Health Organization, 2008b) (Figure 1). In June 2005, an HPAI (H5N1) outbreak occurred in Qinghai Lake of China and caused more than 6,184 dead of migratory birds (Chen et al., 2006a). The Qinghai virus possesses NS, M, PA, PB1 and PB2 genes similar to those of genotype Z. The remaining genes, HA, NA and NP, are closely related to those of a genotype V virus (Chen et al., 2006a, b). Furthermore, an isolate from Vietnam in 2005 was classified in genotype G. This genotype contains PB2 gene resembling to that of genotype W but the remaining genes are closely related to those of genotype Z (Chen et al., 2006b). Additionally, the routine surveillance of poultry in live bird markets in southern China revealed a new strain of virus subtype H5N1 called Fujian-liked virus. From 2005 and 2006, this strain has circulated in the poultry population and become the dominant strain. Serological study showed that most of the sera from vaccinated birds in China failed to neutralize the Fujian-liked virus. The virus caused outbreaks in Hong Kong, Laos and Thailand (Nakornpanom province) in 2006 (Smith et al., 2006a) and in Nongkai province, Thailand, during March 2007.

From the first outbreak in 1997 to 2008, nine distinct clades of HPAI (H5N1) viruses are currently circulating in poultry population in Asia (World Health Organization, 2008b). The clade numbers were assigned in the phylogenetic tree according to the consultation among representatives of OIE, FAO and WHO (World Health Organization, 2008b). The viruses isolated from Hong Kong in 1997 are classified in clade 0. Clade 1 includes isolates from Thailand, Vietnam, Laos, Cambodia and Malaysia. The HPAI viruses from Indonesia and Quinghai are clustered in clade 2.1 and 2.2, respectively, while those from Japan and Korea are in clade 2.5. The Fujian-liked viruses form a separated branch defined as clade 2.3.4. The antigenic properties of viruses in each clade are possibly different and may affect the protective efficiency of HPAI (H5N1) vaccine.

Role of vaccination in evolution of virus

Influenza A virus is RNA virus which its polymerase enzyme lacks proofreading activity, resulting in replication mistake every 10^{-4} nucleotides per se. Thus, high mutation rate, large population size and short generation time are inherited properties of the RNA viruses (Grenfell et al., 2004). Population of viruses in a host then appears as quasi-species. The quasi-species which are the most fit in a particular environment would become the predominant population. Fitness of the virus at both intra- and inter-host levels is also affected by host immunity and neutral epidemiological process, respectively. Consequently, strain of viruses is influent by mutation whereas survival of viruses depends on the existing immunological and epidemiological pressures.

Host immune response against HPAI (H5N1) is usually acquired from natural infection or vaccination. Immune pressure would select only escaping mutants which possess alteration of key amino acids within antigenic sites of HA protein (antigenic drift). Immunity provoked by infecting or vaccinating with the previous strain is unable to neutralize the escape mutants which may initiate new outbreaks. Prolong and wide use of vaccination in animal population may enhance the selective pressure and drive the mutation at phenotype level resulting in rapid antigenic drift (Grenfell et al., 2004; Lee et al., 2004). Theoretically, utilization of a good quality vaccine producing from the homologous strain and containing optimal concentration of antigen would be able to diminish amount of surviving viruses to be less than the infective dose and thus interrupt further transmission (Webster et al., 2006). Practically, use of vaccination alone without other stringent control measures and monitoring for infection and flock immunity may push the disease situation toward endemic instead of eradication (Capua and Marangon, 2004) since silent spreading of the HPAI virus may occur (Savill et al., 2006). Thus, if routine vaccination is included in the HPAI (H5N1) control program, monitoring flock immunity, surveying disease status to reveal new variants and annually analyzing appropriate seed vaccine virus should be considered to prevent new outbreaks.

In addition to genetic drift, one might question if vaccination in poultry would accelerate transformation

of HPAI (H5N1) virus to be a human influenza virus. Three following steps are required for the virus to successfully cross species: close contact between virus and host, virus-host interaction, and adaptation of virus to the new host (Kuiken et al., 2006; Webby et al., 2004). In the first step, certain amount of viruses, at least equal to an infective dose, must gain an opportunity to contact with a new host. Usually, people become infected when the person contacts directly to large amount of the viruses from sick birds or highly contaminated environment. In the case of HPAI (H5N1), most patients and infected mammals had heavily exposed to the virus by inhalation or ingestion (Beigel et al., 2005). In contrary, health care workers exposed to substantial amount of the HPAI (H5N1) viruses from the patients were not infected (Apisarnthanarak et al., 2005).

After the virus becomes in close contact to host, the viruses must cross biological barriers (Kuiken et al., 2006; Webby et al., 2004). Firstly, they have to traverse the natural constitution of host innate immunity such as mucus, cilia movement on epithelial cells and local macrophage. Subsequently, the viruses must interact specifically to a specific molecule called receptor on the cellular surface for entering into host cells. The receptor for the HAPI (H5N1) virus is sialyl á(2,3)-galactose oligosaccharide side chain while that of human influenza virus is sialyl á(2,6)-galactose linkage. However, the avian virus receptor does present on type II pneumocytes lining human's lower respiratory tract (van Riel et al., 2006). After entry, the viruses begin the replication process which may require putative host factor(s) to enhance viral polymerase activity. The virus possessing polymerase proteins compatible with the host cellular factor(s) would replicate vigorously and produce large amount of virus progenies which subsequently release from cells and be shed from host body. In deed, amino acid position 627 on PB2 may involve in adaptation of avian influenza viruses to mammalian cells (Subbarao et al., 1993). Substitution of glutamine to lysine at this position changed a non-lethal HPAI (H5N1) virus (A/Hong Kong/486/97) to a virulent virus that could kill mice in 5 days (Hatta et al., 2001). Additionally, PB2 of HPAI (H5N1) virus isolated from mammalian host including human and felids contains lysine at residues 627 (Amonsin et al., 2006; Hatta et al., 2001). Thus, lysine 627 on PB2 is possibly associated with a cellular protein to promote replication of virus in mammalian cells.

In the next step, the progeny viruses require further adaptation to achieve high level of transmission among new species and maintain their niches in the novel population. Adaptation of viruses to the new host may occur prior to or after the leaping cross species (Kuiken et al., 2006). It is obvious that the HPAI (H5N1) viruses are able to infect mammals including people (World Health Organization, 2008a); however, they lack ability to transmit efficiently among people. Thus, adaptation of the virus in a new host would allow selection of variants that inherit transmissibility among human population. Experimentally, adaptation of avian influenza viruses to mammalian cells occurred when passed these viruses in mammalian cell lines for couple rounds (Matrosovich et al., 2000). These adapting avian viruses had mutations of amino acids within their receptor binding sites that changed the viruses from avian restricted to mammalian specific. Likewise, allowing replication of the avian viruses in human cells may facilitate similar type of adaptation. A recent study showed that a number of sequences amplified from a human isolate of HPAI (H5N1) virus contained alteration of amino acid in the receptor binding pocket (Auewarakul et al., 2007). These HA acquired ability to bind both avian and human receptors. Therefore, one of the measures to avoid the next flu pandemic is to prevent the transmission of HPAI (H5N1) viruses to human or other mammals.

Inter-species transmission will not occur when the viral load in the environment is less than the infective dose. Vaccination in poultry with a high quality vaccine will reduce susceptibility of animals to infection and much decrease virus shedding (after expose to the field virus) which in turn resulted in decreasing amount of viruses in the environment. Thus, properly vaccination would indirectly prevent adaptation of viruses in the new host. Although the antigenic drift on the HA molecule may occur after vaccination, these mutations usually take place within antigenic site, not the receptor binding site (Grenfell et al., 2004; Lee et al., 2004; Stevens et al., 2006). Up to date, most of the HPAI (H5N1) viruses isolated from birds, human and felids still have avian virus properties (Chen et al., 2006b; Amonsin et al., 2006; The World Health Organization Global Influenza Program Surveillance Network, 2005; Smith et al., 2006a, b). Their HA proteins contain two amino acids within the receptor binding site specific to avian cells, glutamine at position 222 and glycine at position 224. Please note that quasi-species of the HPAI (H5N1) viruses including A/Hong Kong/213/97 capable to bind both avian and human receptors were reported (Shinya et al., 2005; Kongchanagul et al., 2008). However, the reassortment between human and the HPAI (H5N1) viruses has not been documented. Indeed, the immune pressure influences the development of antigenic drift but does not relevant to antigenic shift (Grenfell et al., 2004). These evidences confirm that vaccination in

poultry is not associated with alteration of host specificity. In another word, the immune pressure does not increase probability of transformation from avian to human viruses.

Role of vaccination in disease prevention and control

Fundamental of disease or outbreak control is to avoid contact between susceptible hosts and sources of infection as well as decrease the concentration of viruses to the level that transmission is disrupted. Although, aerosol transmission may involve in influenza virus infection, transmission via large-droplet or droplet nuclei which requires close contact among hosts and infectious sources is considered as the major mode (Tellier, 2006). In poultry production, there are various sources of infection including reservoirs, infected birds and contaminated fomites such as utensils, vehicles as well as workers. The most important reservoir for HPAI (H5N1) viruses in Asia is domestic ducks (Hulse-Post et al., 2005) while wild ducks and geese may be reservoirs for perpetuating the viruses in Lakes of China (Chen et al., 2006a, b; Smith et al., 2006a). Migratory birds such as ducks, geese and swans are thought to be the spreaders carrying the disease to Eurasia, Europe and Africa (Gilbert et al., 2006; Keawcharoen et al., 2008). Ideally, preventing the viral transmission is possible by rising the poultry in a close compartment with strengthen biosecurity in which infecting sources are absolutely excluded and contained. In fact, such compartment is not easily to acquire and if acquired, it is difficult to sustain. In the low biosecurity compartment, exposure to the viruses is unavoidable; thus, appropriate use of vaccine will increase resistance of these birds to the disease. Although vaccinated birds can be infected by the viruses, they shed much less amount of the virus into the environment (Swayne et al., 2001; Liu et al., 2003; Qiao et al., 2003).

A transmission experiment demonstrated that inoculated chickens with a homologous HPAI (H7N1 or H7N3) virus at two weeks after vaccination decreased shedding of the virus to the level that could not be transmitted to susceptible chickens kept in the same cage (Van der Goot et al., 2005). Thus, continual transmission of viruses is unlikely to occur. Additionally, maintenance of high biosecurity situation in the clean compartment would become feasible when the viral load in the vicinity is low (Capua and Marangon, 2006). In the endemic area, vaccination in poultry surrounding the compartment would certainly decrease amount of the viruses in the environment which in turn lower the risk of introducing viruses into the compartment. In this scenario, vaccination acts as a second protective layer for the clean compartment (Capua and Cattoli, 2007) During pan-epizootic outbreak, especially, when density of poultry population is high, vaccination may be implemented not only in low but also high biosecurity sectors to decrease the outbreak (Maragon et al., 2007). In this situation, vaccination or strengthening of biosecurity alone is not able to prevent spreading of the viruses since the transmission rate of HPAI viruses is very high (Savill et al., 2006; Van der Goot et al., 2005).

Disadvantages of vaccination

The vaccination seems to be an effective mean to combat the HPAI (H5N1); however, it is not the magic tool and has some drawback. Most of influenza vaccines including high quality products when used properly prevent clinical signs but not infection (Swayne et al., 2001; Liu et al., 2003; Qiao et al., 2003). In fact, the HPAI (H5N1) viruses in the natural environment are not homogeneous population. Some of which may possess different antigenicity. Thus, after exposure to the field viruses, vaccinated birds become infected without disease signs but excrete small amount of the viruses from respiratory tract and/or in feces. The insignificant quantity of shed viruses may cause silent spreading of the disease between flock, when biological security is compromised (Savill et al., 2006). At high level of protection, fewer birds become infected and outbreak is harder detected.

Secondly, both infected and vaccinated animals produced indistinguishable immune response to the viral antigens which interfere to the differential detection. Thus, co-mingling 1% of immunologically naïve birds in the flock may help unmasking the silent infection and making the detection feasible. When possible, the differential diagnosis capable to discriminate between infected and vaccinated animals (DIVA) may apply for routinely monitoring system to spot on the infected flock (Capua and Marangon, 2006). Therefore, stringent biosecurity plus rapid detection and removal of infected birds are required for successful vaccination program (Savill et al., 2006).

In addition to the aforementioned circumstance, inappropriately use of vaccines is certainly unable to cease the outbreak. Low quality vaccines containing suboptimal concentration of antigens and/or manufacturing poorly may inhibit clinical signs but vaccinated and infected birds shed large amount of the viruses; hence cannot block further transmission (Webster et al., 2006). In addition, incorrectly handling vaccine such as keeping at suboptimal temperature during delivery, administrating to unsuitable routes and giving with improper dose to birds will affect on amount of available antigens for inducing immune response. These mal-practices would cause similar results as utilizing low quality vaccine.

On the other hand, if vaccine is used properly but coverage of vaccination is low, it is implausible to stop the disease outbreak. Transmission efficiency of the HPAI is quite high with reproduction ratio (R_0) more than three (Savill et al., 2006; Van der Goot et al., 2005). Therefore, vaccine coverage must be over 90% and all of the vaccinated birds must be protective to decrease R_0 to be less than one. At this ratio, the transmission is unlikely to occur and thus outbreak is inhibited. However, 90% vaccine coverage is practically difficult in non-farm system.

Moreover, vaccination in poultry requires large number of resources including vaccine, manpower and expense. Number of vaccine doses should be sufficient for all poultry to be vaccinated as planned. Large amount of the expense and requirement of the manpower include not only for vaccination but also for vaccine managements, vaccination campaign, monitoring of flock immune status, as well as disease surveillance.

Conclusion

HPAI (H5N1) virus is highly contagious and has been implicated in fatal human infection. Up to date, the virus possesses avian preference; however, its evolution is undergoing. Genetic variation of the virus occurs naturally and adaptation from avian to human virus is unlikely to associate with vaccination in poultry. Since HPAI (H5N1) is highly contagious to birds and causes a fatal disease in human, effective control measures are required to defeat the virus transmission. The control strategy should exploit combination of all measures such as appropriate vaccination, strong disease monitoring and surveillance plus strengthening of biosecurity. Biological security also includes control of animal movement, destruction of infected poultry and poultry at risk of infection and properly disposal of carcasses and infected materials (Food and Agriculture Organization and World Organization for Animal Health in collaboration with World Health Organization, 2005). The control strategy may comprise of short and long term plans. In the short term, vaccination and increasing biosecurity are aimed to confine the infected population in the endemic area and inhibit spreading of the viruses to the clean compartments. In the next step, the strategic plan intends to increase number of high biosecurity compartments and decrease the endemic area as well as define areas at risk of infection. The long term goal is to eradicate the disease from endemic regions with limiting to stopping vaccination.

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Another Thai province reports H5N1 outbreak

Officials in Thailand said that H5N1 avian influenza struck poultry in Uthai Thani, the second province to report the virus in less than a week. Sakchai Sriboonsue, Director-General of the province's Livestock department said that laboratory tests detected the H5N1 virus in chickens from a backyard farm after several of the birds died. Uthai Thani is in northern Thailand. On Nov 11, Thai officials reported an outbreak in Sukhothai province, the country's first in about 10 months. Sukhothai is located north of Uthai Thani. In their report to the World Organization for Animal Health (OIE) detailing that outbreak, Thai authorities said five birds at the affected household were first thought to have died from *Escherichia coli* infections and intestinal parasites. However, samples submitted to the National Veterinary Laboratory in Bangkok revealed the H5N1 virus. The report said authorities were still investigating the source of the virus. In other developments, Thailand's public health ministry has put nine northern provinces under special watch for avian influenza, namely Phitsanulok, Tak, Phetchabun, Sukhothai, Uttaradit, Nakhon Sawan, Uthai Thani, Kamphaeng Phet, and Phichit.
http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/nov1308birds-br.html.