Avian Influenza: Update

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Abstract

Avian influenza is an important zoonotic disease that has become endemic in Southeast Asia and in the case of highly pathogenic H5N1 has caused significant human mortality and morbidity. Avian influenza virus (AIV) infections may be asymptomatic or present as mild conjunctivitis in humans; however, H5N1 infections may present as severe pneumonia progressing to acute respiratory disease syndrome, multiorgan dysfunction syndrome and death. Since 2003, there have been a total of 436 cases and 262 deaths due to H5N1 infections. The number of cases has decreased steadily since 2006; however, the emergence of multiple genetically distinct sublineages of H5N1 has continued. These emerging sublineages display varying levels of drug resistance and in some cases an increased preference for binding to human α 2,6-linked sialic acid cellular receptors. Though H5N1 has not shown efficient transmissibility between humans, the rapid evolution of the virus presents a concern for the emergence of a virus with this capability. Here we review the epidemiology, host range, clinical features, prophylaxis and treatment options, and preventative measures recommended for AIV with an emphasis on H5N1.

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Introduction

Influenza A, B and C viruses belong to the Orthomyxoviridae family and are responsible for both seasonal outbreaks and pandemics in humans. Where type B and C influenza viruses are limited to humans, the influenza A viruses are found in a variety of hosts, both avian and mammalian. Seasonal influenza, caused by those viruses possessing the hemagglutinin (HA) types H1, H2 or H3, is responsible for 3–5 million severe cases per year as well as 250,000–500,000 deaths per year [1]. In addition to seasonal influenza, H1, H2 or H3 viruses, either of or sharing avian origin, have been responsible for each of the major pandemics observed in the last century, including the 1918 ('Spanish flu'), 1957 ('Asian flu'), 1968 ('Hong Kong flu') and 2009 ('swine flu') pandemics. In addition to these pandemic strains of influenza, avian influenza viruses (AIVs) of the H5 and H7 types have emerged, causing devastating outbreaks in poultry and direct infections in humans. In this review, we discuss the present status of human infection with AIV and the impact of the evolution of H5N1 viruses on current treatment and vaccination options.

Influenza A Viruses

Influenza viruses are classified into subtypes by the antigenic characteristics of the HA and neuraminidase (NA) glycoproteins which are found on the surface of the

Fig. 1. Structure of the influenza virus. M = Matrix; PA, PB1, PB2 = polymerases.

virus. AIVs are further classified by pathotype, which is determined by the level of mortality and disease caused by the viruses when intravenously inoculated in chickens, and designated as either highly pathogenic influenza (HPAI) or low pathogenic influenza (LPAI) [2]. HPAI viruses are additionally distinguished from their LPAI virus counterparts by the presence of a multibasic cleavage site on the HA protein, which allows maturation of the virus in a considerably broader tissue range due to an increased number of proteases capable of cleaving at this site [3].

AIV Structure and Features

The AIV genome is organized into 8 negative-sense-oriented RNA segments which are tightly bound by the nucleoprotein (NP) forming the ribonucleoprotein (RNP) core (fig. 1). The RNP is bound to the heterotrimeric polymerase complex consisting of PA, PB1 and PB2 proteins, and is also associated with the matrix M1 protein which underlies the host-derived lipid bilayer envelope [4, 5]. The M1 protein is associated with the nonstructural protein NS2 as well, which is important for the trafficking of the RNP to the cell nucleus during replication. The virus envelope is spanned by the matrix M2 ion channel protein, which functions during viral entry by promoting

viral uncoating, and is punctuated by the two surface glycoproteins HA and NA (the protein responsible for viral release) [5]. Additional nonstructural proteins are encoded by the virus genome and are expressed once the virus has achieved entry into the cell: the NS1 protein, which is involved in the modulation of the host immune response, and the PB1-F2 protein, which plays a role in apoptosis [6, 7].

As a consequence of the genome organization and its replication, genetic variation is accomplished by two major mechanisms: genetic drift and shift. Genetic drift and shift act to generate novel influenza sequences and gene combinations and are a function of the polymerase complex, which lacks proofreading capability, and of the segmented nature of the virus, respectively [8–10]. Genetic drift is the accretion of point mutations over time due to errors in ribonucleotide insertion of the polymerase complex during replication; this mechanism is generally believed to be responsible for the accumulation of polybasic amino acids at the cleavage site of HA, resulting in the transition of a low pathogenicity HA to a high pathogenicity HA, the mechanism for the generation of antigen escape viruses, as well as the reason why a new vaccine must be created each year. Genetic shift is the process by which viral gene segments are reassorted. This process occurs when two distinct viruses coinfect a single cell and replicate, and during virus packaging the RNA segments become shuffled, resulting in new gene constellations composed of mixed segments derived from the coinfecting parental viruses. Genetic shift is believed to have been the driving force behind the generation of the combination of gene segments that made up the viruses responsible for the 1957 Asian flu and the 1968 Hong Kong flu pandemics. In addition to these two predominant mechanisms of genetic variation, influenza viruses are also capable of recombination to a lesser extent, as evidenced by the generation of a high-pathogenicity H7N3 virus from a low-pathogenicity virus by the recombination of the HA and NP genes [11]. In these cases, only a portion of the HA and NP genes had larger genetic changes (recombination) that led to a bigger jump in genome change.

Host Range and Specificity

H5N1 has been isolated from an expansive range of hosts including both avian and mammalian species. The avian host range is particularly wide, including hundreds of wild bird species with members such as ducks, geese, pigeons, parrots and ostriches; domesticated gallina-

aceous species such as quail, pheasants, and particularly turkeys and chickens are susceptible to H5N1 [12–16]. Of these avian hosts, free-flying waterfowl and shorebirds are believed to be the major reservoir, harboring all 16 HA and 9 NA, and have been recognized as asymptomatic carriers of the virus [17–20]. The mammalian host range is not quite as extensive; to date H5N1 has been isolated from domestic cats, Owston's civets, leopards, tigers, dogs, stone martens, pigs, humans and, recently, a plateau pika (GenBank accession No. FJ390061) [12–16, 21–26]. In addition to these naturally occurring infections, ferrets, mice, guinea pigs and cynomolgus macaques have been shown to be susceptible to experimental H5N1 infection in a laboratory setting [27–31].

The host range of influenza viruses is generally believed to be restricted by the type of sialic acid linkage to the terminal galactose on host glycoproteins, or glycolipids where avian viruses preferentially bind sialic acids with $\alpha 2,3$ linkage and human viruses bind $\alpha 2,6$ -linked sialic acids [32, 33]. The distribution of these sialic acid linkages may differ within and amongst avian, mammalian and human hosts [34]. In ducks, $\alpha 2,3$ -linked sialic acids predominate on the surface of epithelial crypt cells of the intestine, supporting enteric replication of avian influenza [35], whereas in humans, $\alpha 2,6$ -linked sialic acids are found on the surface of epithelial cells along the nasopharynx, trachea and bronchi, supporting an upper respiratory tract infection with human influenza viruses [36]. However, the conjunctiva and lower respiratory tract of humans express the $\alpha 2,3$ -linked sialic acid, providing portals of entry for AIVs such as H5N1, leading to a more damaging lower respiratory tract infection [35, 37]. In some instances H5N1 viruses have demonstrated a mild affinity for $\alpha 2,6$ -linked sialic acids in addition to $\alpha 2,3$ -linked sialic acid, suggesting an increased ability for H5N1 to bind human sialic acid receptors [38–41]. Swine tracheal epithelia exhibit a mixed population of $\alpha 2,3$ - and $\alpha 2,6$ -linked sialic acids and are therefore capable of binding both avian and human influenza viruses. These distributions of sialic acid linkages therefore dictate to a large extent the host range of H5N1 viruses, by both the increased tendency to bind either linkage type and by the distribution by location within a host. While HA binding of host receptors is crucial for viral attachment, it is not the definitive restriction for the host range, as the viral polymerase complex must be compatible with the host cellular machinery for productive replication to occur [42, 43].

Epidemiology and Clade Nomenclature

H5N1 emerged in Hong Kong, China, in 1996, causing a significant HPAI outbreak in poultry in 1997 and resulting in 18 recorded human cases, 6 of which were fatal that year [44]. However, no human cases were documented from 1998 to 2002, until January 2003, when one family member died of H5N1 infection, a second member recovered and a third member died of a severe respiratory disease that was not diagnosed [45]. In the course of the year 2003, large poultry outbreaks were confirmed in Korea and went unconfirmed in other parts of Asia, poultry outbreaks occurred in Vietnam and Thailand, and H5N1-infected captive big cats in Thailand were reported at the close of 2003 and beginning of 2004 [23, 46]. In 2004, H5N1 continued to circulate in Vietnam and Thailand, resulting in widespread poultry, wild bird and human outbreaks with 20 confirmed human deaths in Vietnam and 12 in Thailand [47–50]. Between 2005 and 2007, the virus spread to 14 countries including countries in Europe, Africa, Asia and the Middle East, resulting in devastating poultry outbreaks and a sum of 181 human deaths [51–56]. During this period, the first documented case of an AIV transmitting between humans was confirmed, along with multiple other suspected but unconfirmed transmissions between family members in both Vietnam and Indonesia [57–60]. By the end of 2006, the number of both cases and deaths peaked, with the majority of both observed in Indonesia, followed by a decline in both by the end of 2007 [55, 61]. This trend of decreasing cases and deaths reported continued during 2008, coinciding with a decrease in the total number of countries affected, with 6 countries still reporting cases (Bangladesh, Cambodia, China, Egypt, Indonesia and Vietnam) and 4 reporting deaths (China, Egypt, Indonesia and Vietnam) [55]. By the end of 2008, 44 cases and 33 deaths were confirmed, with Indonesia remaining the main contributor of reports (table 1) [55].

As of July 2009, there are currently 41 confirmed human cases and 12 deaths due to H5N1 infection, which is the fewest seen since 2003 [55]. These numbers have drastically decreased in comparison to the peak case and death count in 2006, which were 115 and 79, respectively. Year 2009 cases and deaths have both been reported in China, Egypt and Vietnam, with Egypt registering most cases of H5N1 infections with 30 reports, while China and Vietnam reported 7 and 4 cases [55, 62, 63]. All three countries have reported 4 deaths due to H5N1, bringing the total number of deaths due to H5N1 to 262 [55].

Table 1. World Health Organization fatality rates by country (n)

Country	2003		2004		2005		2006		2007		2008		2009		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	8	5	0	0	0	0	0	0	8	5
Bangladesh	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Cambodia	0	0	0	0	4	4	2	2	1	1	1	0	0	0	8	7
China	1	1	0	0	8	5	13	8	5	3	4	4	7	4	38	25
Djibouti	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	25	9	8	4	27	4	78	27
Indonesia	0	0	0	0	20	13	55	45	42	37	24	20	0	0	141	115
Iraq	0	0	0	0	0	0	3	2	0	0	0	0	0	0	3	2
Laos	0	0	0	0	0	0	0	0	2	2	0	0	0	0	2	2
Myanmar	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Nigeria	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1
Pakistan	0	0	0	0	0	0	0	0	3	1	0	0	0	0	3	1
Thailand	0	0	17	12	5	2	3	3	0	0	0	0	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	0	0	0	0	12	4
Vietnam	3	3	29	20	61	19	0	0	8	5	6	5	4	4	111	56
Total	4	4	46	32	98	43	115	79	88	59	44	33	38	12	433	262

Courtesy of the World Health Organization [49]. Counts current as of July 2009. Interestingly, the case fatality rates by country vary greatly, with China at 36.4%, Egypt at 11.8% and Vietnam at 50.0%. This may reflect differing case fatality rates of virus clades as clade 2.3.4 viruses are circulating in China and Vietnam and clade 2.2 viruses are circulating in Egypt. However, additional studies are needed to assess clade relationship with case fatality rates.

In a joint effort of the World Health Organization (WHO), World Organization for Animal Health and Animal Welfare, and Food and Agriculture Organization, a unified nomenclature system has been devised to adequately determine the sublineages of H5N1 responsible for outbreaks. Previously, an assortment of different classification systems was used to designate branches of evolutionarily distinct strains of H5N1, resulting in confusion and discontinuity. The current system is made up of 10 distinct clades with further subclades designated by decimal points to indicate additional sequence divergence from ancestral viruses. At this time, the clades capable of infecting humans are limited to clades 0, 1, 2 and 7, where clade 2 is made up of several sublineages that continue to circulate and differentiate [52]. Phylogenetic studies have been conducted to determine the clade and subclades of previously and currently circulating H5N1 viruses capable of infecting humans in several countries and regions, the results of which can be seen in figure 2 [52, 64]. The first strain of H5N1 to emerge in 1997 is of clade 0 origin, the 2003–2005 human outbreaks in Vietnam and Thailand are attributed to clade 1 viruses, and clade 2.1 viruses are responsible for Indonesian outbreaks. The most geographically widespread H5N1 strain, the clade 2.2 viruses, has spread from Qinghai Lake in China throughout parts of western Asia, Europe,

the Middle East and Africa. Accordingly, clade 2.3 viruses are predominantly isolated in southern China, and clade 7 viruses are similarly predominantly isolated in China [53, 54, 56, 65, 66]. Since 2008, the currently circulating clades of H5N1 infecting humans are 2.3.2 (China), 2.3.4 (China and Vietnam), 2.2 (Egypt), 2.1 (Indonesia), 1 (Cambodia) and 7 (China) [67].

Clinical Features of Disease

H5N1 infection results in a wide spectrum of presentations ranging from asymptomatic to mild symptoms of flu-like illness (dyspnea, coughing, fever) to severe pneumonia, acute respiratory disease syndrome (ARDS) and multiorgan dysfunction syndrome (MODS). For the majority of patients (median age 20 years, range 2 months to 90 years) first symptoms generally appear in the range of 2–8 days after exposure to sick or dead birds [52, 68]. The

Fig. 2. Phylogenetic tree modified from WHO [49] and Abdel-Ghaffar et al. [52]. Locations of previous and currently circulating clades. Currently, clade 2.3.4 viruses are circulating in China and Vietnam, and clade 2.2 viruses are circulating in Egypt.

most common symptoms occurring in nearly all cases are a fever of more than 38°C, shortness of breath, and a cough [69]. Pneumonia leading to ARDS, MODS and death is the principal finding [44, 47, 50]. Complications may include Reye's syndrome, pneumothorax, pulmonary hemorrhage, nausea, vomiting and ventilator-associated pneumonia [50, 51, 69–72]. Laboratory findings include lymphopenia, thrombocytopenia, hypoalbuminemia and elevated serum proteins such as liver aminotransaminases, lactate dehydrogenase and creatine phosphokinase [69]. Lymphopenia and increased lactate dehydrogenase have been associated with a poor prognosis [51, 56, 73]. Symptomatic of severe pneumonia associated with H5N1 infections, thoracic radiographic findings typically include air bronchograms, interstitial infiltrates and lobar consolidation [52, 69, 74]. The clinical course of H5N1-infected patients is characterized by rapid progression, with 68% of patients developing ARDS and MODS within 6 days of onset of the disease [75]. The current overall case fatality rate is 59.2% (range 57–80%) [44, 47, 50]. Once patients have reached the critical care unit, mortality rates are much higher, reaching 90% [75]. The average time of death after onset of the disease is 9–10 days [52].

In patients with high viral loads from tracheal and nasopharyngeal samples, and in patients in later stages of the disease, high plasma levels of cytokines and chemokines, most notably interleukin-6, tumor necrosis factor- α and the interferons, are evident, which is suggestive of the dissemination of the virus. Indeed, viral antigen and/or RNA have been detected in multiple tissues collected post mortem, including lung, upper trachea, liver, small and large intestinal epithelia, macrophages, bone marrow and the brain [68, 76, 77]. The elicitation of high levels of proinflammatory cytokines and chemokines further provokes tissue damage on top of virus-induced tissue damage, as evidenced by postmortem findings such as diffuse alveolar damage and lymphocyte infiltration [78]. Other autopsy findings include acute renal tubular necrosis and atrophy, hepatic central lobular necrosis, disseminated intravascular coagulation and multiorgan damage [78–80].

Unlike seasonal influenza, H5N1 infections are not commonly associated with upper respiratory tract infection; rather they are more likely to cause pneumonia and lower respiratory infection, likely due to the increased expression of $\alpha 2,3$ sialic acid linkages in the lower respiratory tract. H5N1 is also more commonly associated with gastrointestinal symptoms than seasonal influenza, with case presentations including diarrhea,

vomiting and abdominal pain, and with these symptoms more commonly occurring in adolescent than adult cases [74, 76, 81]. The rapid disease progression, development of ARDS and MODS, and eventual death also occur at a higher rate with H5N1 than with seasonal influenza [75].

Diagnosis

H5N1 clinical diagnosis is difficult as infected patients present with symptoms common to other illnesses, making the definitive diagnosis contingent upon viral isolation and/or detection of viral RNA. Identifying factors contributing to avian influenza exposure, such as interaction with sick or dead birds, travel or habitation in endemic areas or contact with individuals known to be infected with AIV, may provide important leads. In recent years, real-time RT-PCR has become the primary method of detecting viral RNA in respiratory specimens, followed by viral isolation. RT-PCR is dependent on the use of primers and probes specific to the viral subtype, and is therefore discriminative between seasonal influenza strains and avian influenza subtypes with a high degree of specificity and sensitivity. Viral isolates may be sequenced, subtyped and analyzed for reassortment, mutations that confer replicative advantage, transmissibility and/or drug resistance, and clade identity amongst other studies.

Besides RT-PCR and viral isolation, the presence of specific antibodies is also indicative of viral exposure, past or present (though not particularly discriminative of which). Serology is considered positive with a standard four-fold antibody titer increase and may be used to complement PCR results. The current gold standard for determining antibodies against H5N1 virus is the microneutralization assay, which requires live virus and biosafety level 3 conditions. Antibodies against H5N1 virus can also be determined by the hemagglutination inhibition (HI) assay utilizing horse red blood cells. These assays are useful in determining seroprevalence in a given demographic and in guiding the targeting of active surveillance for avian influenza. In addition to seroprevalence, microneutralization and HI assays may be used to identify antigenic characteristics of strains of avian influenza, which is important in the formulation of vaccines against common epitopes of currently circulating strains of H5N1. Immunohistochemical staining may be used to determine the presence of a virus in various tissues and the extent to which the virus has spread.

Treatment

Two classes of antiviral drugs are currently available to treat patients infected with AIV, the NA inhibitors (oseltamivir and zanamivir) and the adamantanes (amantadine and rimantadine) which target the M2 ion channel. NA inhibitors are the preferred antiviral treatment as they have less serious side effects and a large proportion of strains of avian influenza have grown resistant to the adamantanes. Irrespective of the antiviral drug used, there has not yet been a controlled study as to the efficacy of these drugs against AIV infections, unlike seasonal influenza infections, which have been studied extensively. The only measure of antiviral responsiveness to AIV infections has been derived from experience during AIV outbreaks. As a corollary of the lack of drug trials, the optimal dosage and duration of antiviral treatment are unknown; however, the WHO recommends that those cases presenting with gastrointestinal symptoms, severe cases and those late in the clinical time course be given high doses and receive treatment for an extended period of time [52, 82].

The adamantanes act to block the activity of the influenza protein M2, which forms an ion channel essential for the acidification and subsequent fusion of the virus to the host endosomal membrane, thus blocking efficient viral entry. However, due to the appearance of HPAI H5N1 clade 1 viruses with Ser31Asn and Leu26Ile substitutions in 2003 in Southeast Asia, the adamantanes have limited use in H5N1 treatment [83, 84]. Emergent strains of H5N1 in 1997 as well as some more recent strains of H5N1 including subclade 2.2 viruses are susceptible to this class of drug; however, the concern that circulating H1 and H3 strains of seasonal influenza (which have largely become adamantane-resistant) will reassort with circulating H5N1, providing resistant M gene segments, has resulted in the recommendation that adamantanes not be used for treatment of H5N1 influenza [85]. Therefore, the adamantanes have been relegated to a prophylactic role in cases where the virus is known to be susceptible to the drug.

NA inhibitors prevent viral release during replication by blocking NA-mediated cleavage of sialic acids on the surface of the cell, thereby halting subsequent viral progeny from budding off the infected cell. This class of drug has been studied for both prophylaxis and treatment of human seasonal influenza subtypes H1, H2 and H3, as well as influenza B. HPAI H5N1 studies in animal models have demonstrated that NA inhibitor treatment increased survival rates and decreased viral loads [86]. Oseltamivir

has been used to treat avian influenza cases including subtypes H7N7 and H5N1 with decreased nasal secretions of the virus being reported for patients infected with H5N1 [36, 87–89]. Other reports from Egypt, Indonesia and China indicate that oseltamivir treatment resulted in increased survival and prevention of infection in patients infected with HPAI H5N1 [52, 78, 90]. While resistance to NA inhibitors is not as pronounced in comparison with the adamantanes, resistant viruses have arisen prior to and during treatment for clade 1, subclade 2.2 and subclade 2.3.4 viruses [87, 91, 92]. The clade 1 viruses became resistant to oseltamivir when they acquired the His274Tyr mutation during the treatment of two fatally infected patients [87]. An Asn294Ser substitution in another clade 1 virus was reported to confer resistance to oseltamivir during treatment, whereas isolates of subclade 2.2 viruses were reported to possess the Asn294Ser substitution prior to the infection of patients [68, 92]. In addition to these clinical reports, in vitro studies indicate that currently circulating subclade 2.3.4 H5N1 have decreased susceptibility to oseltamivir and zanamivir; however, they remain sensitive to adamantane treatment [91].

Thus far, experience with the NA inhibitors has shown that the timing of treatment is vital in effecting a positive outcome. Treatment of human influenza viruses early in the clinical course of infection is associated with increased survival, with treatment within 48 h of the infection being optimal [93, 94]. Since many patients are admitted several days after infection with AIV, NA inhibitor treatment of patients infected with H5N1 generally occurs later (4.5–9 days after infection), with reports in Southeast Asia indicating that early treatment appears to improve survival [47, 95]. These reports are also suggestive of a wider window of therapeutic efficacy for oseltamivir as protection from human influenza virus infection is usually decreased when it is administered beyond 60 h after infection onset [93, 94].

Other treatments include combination therapy with antivirals, antiinflammatory therapy and immunotherapy. Currently, there is only one instance of combination antiviral therapy resulting in the survival of the patient [96]. Both oseltamivir and rimantadine were used in this case, the outcome of which warrants further studies in combination therapy as its use would be suitable for those cases where intermediate resistance has arisen (such as infections with subclade 2.2 and 2.3.4 viruses). The usage of ribavirin in conjunction with oseltamivir reduced viral replication in mouse organs during experimental infection, and one child late in infection survived after receiving amantadine and ribavirin [95, 97]. Other com-

bination therapies have not yet been evaluated. Hypercytokinemia is thought to contribute greatly to the pathogenesis of H5N1, especially in the respiratory tract, with elevated serum levels of interferon- γ , soluble interleukin-2, interleukin-10 and interleukin-6 being consistent findings [79]. Despite this, immunomodulatory therapy is relatively unstudied. Corticosteroids have been used in some patients; however, the WHO does not recommend their use as they have not shown any benefits and may result in adverse side effects [52]. No studies have been performed to determine the efficacy of antiinflammatory therapy combined with antivirals. Immunotherapy using convalescent serum has shown some promise although this involved only three severely ill patients in China [95, 98].

At advanced stages of the disease when patients have progressed to ARDS and/or MODS, treatment may require supportive care, mechanical ventilation, vasopressor therapy and renal replacement. In cases where pneumonia has progressed to ARDS, noninvasive ventilation is not recommended; however, it is recommended that early intubation be performed in those cases where clinical signs point towards respiratory failure.

Vaccination

In 2007, the FDA approved an H5N1 vaccine manufactured by Sanofi Pasteur Inc. which has been stockpiled for use in the event of a large outbreak, but not for routine prevention of infection. A reassortant clade 1 H5N1 virus, A/VN/1203/04, was engineered by reverse genetics to formulate the unadjuvanted vaccine; however, while well tolerated, the vaccine has a relatively low immunogenicity [99]. In 2008, the FDA approved another vaccine developed by the Center for Biologics and Evaluation and Research, Food and Drug Administration (CBER/FDA) targeting a clade 2.3.4 virus, A/duck/Laos/3295/2006, which is currently being examined for efficacy [100]. In addition to these vaccines, there are multiple ongoing vaccine trials being conducted in both animal models and in humans. Phase I and II trials have been carried out for a reassortant H5N2 live cold-adapted influenza vaccine, which was well tolerated and generated a ≥ 4 -fold increase in titers of specific antibodies and cross-reactivity to HPAI H5N1 in infected macaques [101]. Another phase I trial found that a single dose of a clade 1 H5 expressed in a baculovirus vector, administered intramuscularly, was capable of inducing a ≥ 4 -fold increase in titers of HI antibody in 68% of the subjects [102]. A phase

II study in Australia was conducted with a large randomized group to determine the efficacy of a clade 1 H5N1 split-virus vaccine, and found that high amounts of HA were capable of generating persistent seropositive antibodies ($\geq 1:32$ HI titers) for up to 6 months that also showed some cross-reactivity with currently circulating clade 2 viruses [103]. These studies, while promising, still fall short of eliciting the antibody titers known to be protective in formulating vaccines for seasonal influenza ($\geq 1:40$ HI titers); however, it is unknown whether these levels are the same as those required for protection against AIV. These studies also indicate that the best strategy for vaccine development, whether that be subunit, DNA, whole-inactivated or live-attenuated, is currently unknown. The same can be said for the optimal prime and boost strategy and adjuvant formulation. The rapid evolution of new subclades highlights the challenge that vaccine development faces, as clade cross-protection will likely be an important feature of an effective human vaccine against H5N1.

Infection Control and Prevention

Each wave of human outbreaks of HPAI H5N1 has been preceded by outbreaks in poultry, spurring intensive efforts in the prevention and eradication of infections in poultry as a preventative measure against the spread to humans. These efforts include vaccinations, increased biosecurity and mass culling when infection occurs in a flock. In those countries where infection has not yet spread, surveillance, biosecurity and planning for outbreaks are crucial for the prevention of introduction and the establishment of a state of readiness for AIV outbreaks. These precautions at the regional/country level may reduce the spread of AIV across boundaries; however, precautions at the handling level are absolutely critical for preventing further infections in humans. These precautions have been outlined by the WHO and Centers for Disease Control and Prevention to reduce the incidence of human cases, which can be prevented by taking the proper measures to protect oneself at the health care level. These measures include the use of respirators in the health care setting. Currently, the Centers for Disease Control and Prevention and WHO are recommending a N-95 mask when caring for any individuals with AIV. During high-risk procedures which generate aerosols such as bronchoscopies, noninvasive mechanical ventilation, intubation, humidified oxygen delivery and nebulizer delivery of medication, more advanced respiratory

protection should be considered [104]. This would include the use of a full-face or hood powered air-purifying respirator. Protective respiratory equipment should be worn in conjunction with other personal protective equipment such as impermeable gowns, gloves and face shields. Hand washing with antibacterial soap or alcohol-based washless gels should be performed before and after interaction with patients, with wash stations present in each patient room. Upon admittance, new cases should be isolated in negative pressure rooms with 6–12 air changes per hour. Family and visitor access to patients should be restricted to prevent spread of the virus, and when access is allowed, visitors should be educated as to the importance of limited contact. Health care workers should be vaccinated against seasonal strains of influenza annually to reduce the likelihood of coinfection and the generation of reassortant human-avian influenza viruses. If exposure of a health care worker to an infected individual occurs, antiviral chemoprophylaxis should be on hand. Should health care workers show signs of infection, they should immediately be taken off duty and monitored. With these precautions, the spread of AIV in the health care setting should be minimal, reducing the risk of infection for other patients, family and health care workers.

Conclusion

While cases of AIV infections in humans have diminished since 2006, this in no way reflects a decrease in the danger these viruses pose. With the rapid rate of

evolution exhibited, especially in the development of new subclades of HPAI H5N1, the emergence of a pandemic strain remains a serious threat. The association of certain circulating clades of viruses with drug resistance is another concern that prompts the need for more research and development of antiviral drugs and combinatory strategies to overcome the loss of susceptibility to the adamantanes and the emergence of oseltamivir-resistant strains. Besides antivirals, the assessment of other strategies such as immunotherapy and the use of antiinflammatories in conjunction with current therapies are needed to provide alternatives to conventional treatment should more drug-resistant strains arise. Vaccines in development have shown limited success, though the need for studies regarding subclade cross-reactivity will no doubt become evident as H5N1 subclades continue to diverge. Thus far, the majority of infections have been due to direct contact with sick or dead birds; however, the limited human-human transmission in family clusters presents a major concern should AIV become more transmissible between humans. Indeed, the increased tendency for H5 viruses to bind to human-type sialic acid linkages suggests an enhanced ability to infect mammalian hosts, providing further opportunity for adaptation to those hosts. Despite the evolution of drug-resistant strains, changes in sialic acid binding and emergence of new subclades, the preventative measures taken for infection control as well as knowledge gained from treatment of infections has obviously had some impact on the number of cases and human mortality as these numbers have been in decline over the past two years.

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