Influenza A(H5N1): an overview of the current situation

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Influenza viruses continue to threaten the world with a new pandemic. While currently attention is focused on the newly emerged A(H1N1) virus, the avian influenza A(H5N1) virus is still a cause of concern. Extended research is focused on the genetic evolution of the viruses, as well as their susceptibility to available antiviral drugs. One of the major priorities of the World Health Organization is to develop candidate vaccines, four of which are already licensed for use in the European Union. Since the last influenza pandemic in 1968, our knowledge of the influenza virus and its biology has greatly increased, revealing new avenues in the research for antiviral strategies and the development of effective vaccines.

Introduction

Influenza viruses continue to threaten the world with a new pandemic. While currently attention is focused on the newly emerged influenza A(H1N1) virus spreading around the globe, the avian influenza A(H5N1) virus is still a cause for concern, not only as a threat in itself but also in combination with the new influenza A(H1N1) epidemic. The newly emerged influenza A(H1N1) strain is spreading rapidly to the human population, which indicates sustained human-to-human transmission, compared to the avian A(H5N1) strains which are highly pathogenic, but with limited ability for human-to-human transmission. No one can surmise the effect of an A(H1N1) spread to the countries where A(H5N1) is endemic. For this reason, continuous influenza surveillance and global monitoring of influenza infections is critical at this point.

Since the re-emergence of the A(H5N1) influenza virus in 2003 in Asia, Africa, the Pacific Region, Europe and the Middle East, the virus has become endemic in some countries, and continues to cause outbreaks in poultry. More importantly, it is now causing sporadic human infections that are associated with high morbidity and mortality rates. Evidently, should an avian influenza pandemic occur, the outcome is likely to be very severe. It is thus of great importance to monitor the emergence of such infections both in poultry and in humans, to isolate and characterise the circulating viruses and to invest in antiviral susceptibility testing and vaccine development.

The World Health Organization is coordinating the global response to human cases of H5N1 avian influenza and monitoring the corresponding threat of an influenza pandemic. The cumulative number of cases of A(H5N1) virus infections reported to WHO until 15 May 2009, was 424 with 261 subsequent deaths, accounting for 61% mortality rate (Figure) [1]. 2006 was the year with the highest number of reported cases and a case fatality ratio of 63% [2]. The reported number of cases declined after that, probably reflecting the successful monitoring and detection of infections in poultry and humans. Fatality rates were high in all age groups, but were the highest in persons between 10 and 39 years of age, regardless of their sex. Cases occurred all year round.

Genetic characterisation of circulating viruses

The hemagglutinin sequences of circulating influenza A(H5N1) viruses are classified into distinct clades. Recent human clade 1 infections have been limited to Cambodia, Thailand and Viet Nam. Clade 2.1 viruses have continued to circulate in poultry and have caused human infections in Indonesia, while clade 2.2 viruses have the most diverse distribution, with outbreaks in birds in over 60 countries in Africa, Asia and Europe and human infections in Azerbaijan, Bangladesh, China, Djibouti, Egypt, Iraq, Nigeria, Pakistan and Turkey. Clade 2.3.4 viruses have been responsible for human infections in China, Lao People's Democratic Republic, Myanmar and Viet Nam. Since September 2008, human infections have been limited to China, Viet Nam, Cambodia, Egypt and Indonesia [3].

A number of recent reports highlight the importance of mutations in A(H5N1) avian influenza viruses, indicating that these genetic variations may increase the possibility of a new pandemic. Influenza viruses are inherently unstable, due to their segmented RNA genome and the lack of a genetic proofreading mechanism that allows undetected errors that occur during replication. Since the first documentation of human infection with the A(H5N1) avian influenza virus in 1997, the virus has undergone several changes. These changes have influenced the patterns of virus transmission and have spread amongst domestic and wild birds. Human infections are still considered a relatively uncommon event as the virus does not spread easily from birds to humans or from human to human. Trustworthy prediction of the evolution of influenza viruses cannot be made, as it is almost impossible to identify whether or when the A(H5N1) virus might obtain the characteristics needed to spread among humans and there is also a lack of knowledge as to which specific mutations will allow human-to-human transmission of the virus [4].

Fortunately, the A(H5N1) viruses have not yet demonstrated the capacity for efficient and sustained human-to-human transmission, although limited transmission is believed to be the cause of

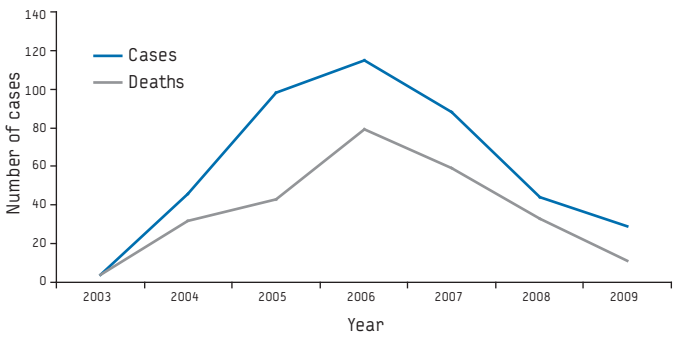
some family clusters of cases [5]. Since those sporadic family clusters of A(H5N1) cases may be the first suggestion of a viral or epidemiologic change, they are being thoroughly investigated in order to determine any direct human-to-human transmission of the virus [6]. Such clusters involving highly probable human-to-human transmission have been documented in Egypt, China, Thailand, Vietnam, Indonesia and Pakistan [7,8]. Studies have also shown a higher prevalence of A(H5N1) antibodies among healthcare workers exposed to A(H5N1) patients in comparison with the prevalence among non-exposed healthcare workers. These findings constitute the epidemiological evidence that A(H5N1) viruses were indeed transmitted from patients to healthcare workers, who then possibly had an asymptomatic infection [9]. Such unconfirmed cases have a potentially huge impact on the case fatality ratio and could indicate that the A(H5N1) virus is probably less lethal than currently assumed.

Furthermore, it was recently observed that undetected A(H5N1) cases may be occurring in Egypt, given the unusual age-specific and sex-specific case incidence and fatality rates, which can be partly attributed to the existence of undetected fatal or non-fatal atypical or asymptomatic human A(H5N1) infections [8]. Asymptomatic human infections with A(H5N1) have been also reported from China, Vietnam, Japan, Thailand, and Korea although limited investigations suggest that the frequency of asymptomatic or clinically mild A(H5N1) virus infection have been rare since 2003 [10]. Most human cases have demonstrated the increased pathogenicity of the A(H5N1) strains.

Tumpey and colleagues, who reconstructed the A(H1N1) virus of 1918, have identified a number of common points between the viruses of Spanish and the avian A(H5N1) influenza. It was concluded that it is especially the polymerase, the hemagglutinin (HA) and neuraminidase (NA) genes that caused the extreme virulence and that the sequences of the polymerase proteins (PA, PB1, and PB2) of the 1918 virus differ by only 10 amino acids from the avian influenza viruses [4]. Human forms of seven out of the 10 amino acids have already been identified in currently circulating influenza A(H5N1) viruses. It is likely that also the other mutations will eventually emerge and make the A(H5N1) virus better suited for human-to-human transmission.

Figure

Human cases (n=424) and deaths (n=261) caused by influenza A(H5N1) virus infection, 2003-2009



Source: World Health Organization. Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO. 15 May 2009 [1].

Another important factor is the change of the HA protein to a binding preference for alpha 2,6 sialic acid, which is the major form in the human respiratory tract. In avian viruses the HA protein preferentially binds to alpha 2,3 sialic acid, which is the major form in the avian enteric tract. It has been shown that only a single amino acid change can result in the change of this binding preference. Altogether it seems that only a few mutations are needed to make the A(H5N1) avian influenza virus a pandemic virus, with possible mortality rates resembling the rates of the Spanish flu, which killed over 40 million people worldwide. Taubenberger et al. have recently showed that the 1918 virus was initially an avian virus, like the A(H5N1) [11].

In February 2004 and May 2005, the influenza A(H5N1) virus was detected in pigs in Viet Nam and Indonesia, respectively, increasing fears of the emergence of new variant strains. Along with the continuing pattern of virus circulation in poultry, the occurrence in swine raised the level of concern about the possible evolution of the virus into a strain with pandemic potential, as pigs may act as a mixing vehicle, in which influenza viruses can recombine with genetic reassortment.

In order to detect any variations that might lead to the development of a potentially pandemic strain, WHO influenza reference laboratories, in cooperation with the national influenza centres of affected countries, are isolating circulating influenza viruses and monitoring their variations with molecular techniques.

Vaccine development

One of the major priorities of WHO is to develop candidate vaccines with representative A(H5N1) viruses from all currently circulating clades. As of February 2009, a number of A(H5N1) reassortants have completed the regulatory approval; these reassortants belong to clades 1, 2.1, 2.2, 2.3.4 and 4 and have been developed by: National Institute for Biological Standards and Control (NIBSC), United Kingdom; Centers for Disease Control and Prevention (CDC), United States (US); Food and Drug Administration (FDA), US; and a consortium of St Jude Children's Research Hospital US, University of Hong Kong, China and National Institute of Allergy and Infectious Disease, US (SJ/HKU/NIAID). A number of reassortant viruses that belong to clades 2.2, 2.3.2 and 7 are prepared and awaiting regulatory approval and there are also two viruses (clade 2.3.4 A/chicken/Hong Kong/AP156/2008-like and clade 7 A/chicken/Viet Nam/ NCDV-03/2008) that have been proposed by WHO for candidate vaccine preparation [3].

The procedure for licensing in Europe is centralised through the European Medicines Agency (EMEA), although national authorisation may still occur at the level of individual countries. To date, there are four licensed pre-pandemic and pandemic vaccines in the European Union. The first approved pre-pandemic vaccine is Prepandrix; it is an A(H5N1) adjuvanted vaccine manufactured by GlaxoSmithKline (GSK) plc, that could potentially protect against a range of different emerging H5N1 strains. The second is Daronrix vaccine, also developed by GSK, which contains inactivated influenza viruses of the A/Viet Nam/1194/2004 (H5N1) strain. When the World Health Organization declares a pandemic, Novartis is approved by EMEA to adapt Focetria vaccine to contain the pandemic strain. In addition, Baxter's A(H5N1) vaccine, Celvapan, is the first approved pandemic vaccine that is cell-cultured based. A number of other countries, including US, Australia, Japan and China, also have licensed products [12].

On 12-13 February 2009, the Department of Initiative for Vaccine Research (IVR) of WHO convened the 4th meeting on "Evaluation of pandemic influenza prototype vaccines in clinical trials". Among A(H5N1) vaccines that have been evaluated, the egg-derived split/subunit, oil-in-water adjuvanted vaccines have demonstrated dramatic antigen–sparing, cross-clade immune responses, and effective priming. The MF59-adjuvanted A(H5N1) vaccine developed by Novartis is being evaluated in phase II trials and Sanofi-Pasteur's AF03-adjuvanted A(H5N1) vaccine is undergoing phase II trials. Other market-approved A(H5N1) vaccine formulations include egg–derived, alum-adjuvanted whole or split virus vaccines in Japan (Biken), China (Sinovac) and Australia (CSL) [13]. The safety and immunogenicity of several A(H5N1) vaccines have been confirmed for both children and the elderly, while the evaluation of prototype pandemic vaccines for these groups is in progress. However, more data need to be accumulated, especially for the very young age groups from six months to three years of age, as in the event of a pandemic, priority immunisations will target the young, the elderly and the individuals that belong to high risk groups [13].

The development, the clinical trials and the licensing process of A(H5N1) vaccines is progressing and it is the responsibility of national authorities to decide on the use of one or more of these for the production of pilot lots of vaccine, depending on the geographical spread, epidemiology and antigenic and genetic properties of A(H5N1) viruses that are circulating in the area. A number of countries have been stockpiling such vaccines. Clinical trials are under way to evaluate vaccination schedules and to detect cross-immunity by vaccines containing viruses from different clades.

Antiviral susceptibility

Until the production of vaccines for prophylaxis against influenza A(H5N1) virus infection is completed, antiviral drugs are the first line of defence. For the treatment of seasonal influenza, two drug categories are currently commercially available, the neuraminidase (NA) inhibitors: oseltamivir and zanamivir, and the matrix protein 2 (M2) inhibitors: amantadine and rimantadine. Early administration of these drugs can reduce the severity and duration of illness from seasonal influenza viruses [14].

Though clinical data related to A(H5N1) infections are limited, it has been shown that early administration of NA inhibitors can decrease the severity of the disease and increase the prospects of survival. In case of a pandemic, the A(H5N1) virus is expected to be susceptible to the NA inhibitors. M2 inhibitors could also be administered against pandemic influenza, however resistance to these drugs may occur rapidly thus reducing their efficacy against the virus. In addition, a high percentage of currently circulating avian influenza A(H5N1) strains is already fully resistant to those drugs [15].

Concerning the NA inhibitors, some of the limitations for many countries are the low production capacity and the economic restrain. Due to the complex and time consuming manufacturing process, the producer of oseltamivir has to build a manufacturing capacity to meet the demands of the global market.

WHO has reserved a certain amount of oseltamivir for use in the first areas affected by an emerging pandemic virus. Based on mathematical modelling studies, the drugs could be utilised for protection purposes at the beginning of a pandemic in order to delay its international spread and gain time to complete the vaccine supply. Influenza surveillance in the affected areas needs improvement, especially regarding the detection of clusters of cases which are closely related in time and place, in order to increase the chances that WHO's rapid intervention will be successful [16,17].

As antiviral susceptibility profiles are changing in various affected areas, combined treatment with both available antiviral drug classes is also a possibility. It is important to clarify whether in a pandemic situation, highly pathogenic A(H5N1) influenza viruses that will have acquired affinity for human rather than avian respiratory epithelium, will also have altered susceptibility to NA inhibitors, which is considered the first line of defence. Relevant studies have not shown such a relation [18].

Resistance to antiviral drugs in influenza viruses can emerge following medication or may result from natural variation. The essential task of the recognition of influenza virus variants resistant to these drugs is accomplished by a select group of the global experts that are members of the Neuraminidase Inhibitor Susceptibility Network, organised by WHO [14,19]. Recent reports on the drug-resistance of the seasonal A(H1N1) virus strains from countries of the northern hemisphere, show a high percentage of strains resistant to oseltamivir. A total of 30 countries have reported resistance to oseltamivir in A(H1N1) viruses, whereas A(H3N2) strains seem to be susceptible to oseltamivir and resistant to adamantanes [20,21].

Basic research on influenza viruses provides a much better understanding of the biology of the virus and offers the possibility of the development of new antiviral drugs [22]. Antibodies against HA that can neutralise virus infection can be potentially developed into effective influenza prophylaxis. Several candidate antibodies against A(H5N1) have been identified, and have found to be effective in neutralising the virus infectivity in tissue culture and experimental animals. Furthermore, short interfering (si) RNAs, that are able to inhibit the expression of specific genes by inducing sequence-specific degradation of target mRNA, have been designed against conserved sequences in the influenza A virus nucleoprotein, polymerase and matrix genes. These siRNAs are able to suppress virus replication and significantly reduce virus yields in tissue culture, and in the lungs of infected mice [22]. In addition, molecules that mimic the structures of the double stranded RNA replicative intermediates, essential for replication, are also considered to be potential drugs against influenza. Such molecules are not produced in the host cell, and their presence in mammalian cells stimulates an antiviral response. Although the in vitro data obtained seem promising, it remains to be established if this approach will be effective in preventing influenza virus infection in humans. Similarly, treatment of cells with chloroquine elevates the endosomal pH, and previous studies have demonstrated its inhibitory effects on influenza virus replication [22].

Since the last influenza pandemic in 1968, our knowledge of the influenza virus and its biology has greatly increased, revealing new avenues in the research for antiviral strategies and the development of effective vaccines. It is clear that the development of vaccines will limit the spread of a pandemic strain and new antiviral strategies will provide new means in countering a new pandemic. However, it is likely that during a pandemic, people that live in many parts of the world will not be able to afford the costs of prevention and

treatment. One of the major challenges in a new pandemic will be the availability of anti-influenza virus vaccines and drugs that can be easily produced on a mass scale, and distributed to all parts of the world.

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