ZOONOTIC DISEASES, HUMAN HEALTH AND FARM ANIMAL WELFARE

Avian influenza

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INTRODUCTION

Avian influenza has the potential to cause rapid and widespread mortality in domestic poultry (i.e. chickens and turkeys). The influenza A virus genes are encoded by RNA rather than DNA, which is encapsulated in the virus as eight segments, each of which encodes one or two proteins. Two of these proteins, the haemagglutinin (HA) and neuraminidase (NA), are each encoded by a separate RNA segment. The HA and NA project from the surface of the virus particle and are responsible for allowing the virus to gain entry into host cells and for newly-produced viruses to exit the cell, respectively. As these proteins are exposed on the surface of the virus, they are the major targets of the body's immune defences. The body recognises the HA and NA as foreign proteins and generates antibodies that circulate in the blood stream for a while and neutralise the virus. On re-exposure to the same virus strain, memory cells rapidly respond to ensure that protective antibodies are generated. However, the HA and NA proteins can be classified into subtypes that are sufficiently antigenically different that antibodies raised in response to one subtype will not neutralise another subtype.

Until recently, there were 17 known different subtypes of HA (H1 to H17) and nine NA subtypes (N1 to N9), all of which had been found in different combinations in wild aquatic birds. As a result, wild aquatic birds, particularly ducks, are seen as the reservoir for influenza viruses from which certain subtypes emerge and occasionally become established in other species. In ducks and gulls, influenza virus primarily replicates in the intestines[1], without causing disease, and is shed in large amounts in the faeces, contaminating water, which is thought to be the primary route of transmission of the virus to poultry. This was illustrated by isolation of an H13N2 virus from turkeys on a range where large numbers of gulls congregated at some nearby surface water; H13N2 virus was also isolated from the water[2]. In 2012, a completely novel influenza virus was isolated from bats in Guatemala, South America[3]. The significance of this discovery is not yet clear, but it serves as a reminder of the unpredictability of influenza viruses.

Usually, influenza infection in poultry causes mild disease, referred to as low pathogenicity avian influenza (LPAI), but two HA subtypes (H5 and H7) can mutate to a highly-pathogenic form (high pathogenicity avian influenza, HPAI) in poultry. Replication of the HPAI virus is not limited to the respiratory tract and it can cause rapid death. In the 40 years between 1959 and 1999, there were 18 reported outbreaks of HPAI[4]. Almost half of these affected more than 100,000 birds each. Most outbreaks of HPAI were brought under control, but the HPAI H5N1 virus that was first recorded in China in 1996[5] continues to circulate to this day and has affected flocks in 63 countries. In the 12 years since the beginning of the current century, there have been a further 11 or more HPAI outbreaks, the latest being an HPAI H7N3 outbreak in Mexican poultry farms that began in June 2012[6].

Risk to human health

If a virus emerges with HA and NA proteins not previously encountered by the majority of people and the virus is able to transmit from person-to-person, then a pandemic (i.e. rapid worldwide spread of a virus to which most people have no immunity) can result, with potentially devastating consequences.

There are several barriers to the transmission of avian influenza viruses to people, the primary one being that the HA of the avian influenza viruses recognise a particular conformation of a receptor protein on host cells (an α2,3-sialic acid linkage) which is largely absent from the cells lining the upper respiratory tract in people. Specific binding of the HA to the relevant receptor is the first stage in the virus gaining entry to the host cell. In order to readily infect people, influenza viruses must adapt (through mutations in the HA gene) to be able to recognise the main receptor protein in the human respiratory tract (α2,6). It has therefore been proposed that avian influenza viruses are more likely to become established in people *via* an intermediary host. As the pig has receptors recognised by both avian and human influenza viruses lining its respiratory tract, it was proposed as a potential 'mixing' vessel in which simultaneous infection of cells with both an avian and a human influenza virus could lead to mixing up of the viral gene segments resulting in a novel combination of HA and NA genes[7].

The most notable pandemic in human history is that of 1918-19, which is estimated to have killed 50 million people. Genetic analysis of fragments of the 1918 virus obtained from preserved tissues of victims of the pandemic has suggested that the virus was entirely avian in origin, but whether the virus was introduced into the human population directly from birds or *via* pigs is unknown[8]. A further two pandemics occurred in the twentieth century – one caused by an H2N2 virus in 1957 and one by an H3N2 virus in 1968[9]. These two pandemic viruses appear to have arisen by reassortment between avian and pre-existing human viruses[10].

An outbreak of HPAI H5N1 in 1997 during which 18 people were hospitalised and 6 died demonstrated that avian viruses can be transmitted directly from poultry to people[11]. Those patients that contracted avian influenza were exposed to a large dose of virus from direct contact with infected chickens. Fortunately, to date, the H5N1 virus has not developed the capacity to transmit effectively from person-to-person. This is believed to be because replication of the virus is restricted to deep within the lungs where there are some of the receptors recognised by the avian influenza virus[12], therefore there is much less release of the virus into the atmosphere by coughing and sneezing compared with seasonal human influenza. Nonetheless, the virus is known to have infected 607 people, of whom 358 died, giving a case fatality rate of around 60%[13].

HPAI viruses are rarely transmitted from poultry to people, but the occurrence seems to be on the increase in line with increasing numbers of reported outbreaks of HPAI among poultry. Since 1997 and the first outbreak of the H5N1 HPAI virus, there have been outbreaks of an H7N7 HPAI virus in the Netherlands in 2003 and of HPAI H7N3 in Canada in 2004 with virus transmission to people. In the 2003 outbreak, infection was confirmed in 89 people; the majority suffered from conjunctivitis, a few had typical signs of influenza infection and one person died as a result of respiratory disease[14]. During the 2004 outbreak, 57 people suffered from conjunctivitis and respiratory illness and influenza infection was confirmed in two of them[15].

Influence of farming practice

Intensive farms concentrate large numbers of animals close together. They also tend to be consolidated in specific geographical areas. They may be close to large cities that they supply or in regions where cereal crops, used for poultry feed, are cultivated, as is the trend in the UK.

Pigs also have cereal-based diet and are a cheap protein source, which can lead to co-localisation of intensive pig and poultry units[16], potentially enhancing the risk of transmission of avian influenza to pigs in which reassortment may occur. There may also be minimal genetic variation among the birds used to stock intensive farms as there are just three large companies at the pinnacle of the breeding pyramid.

Although housing animals indoors may reduce the risk of a new virus being spread on the wind and introduced into a facility from other facilities or wild birds in the vicinity, once a virus is inside an animal house, crowding of animals will increase the likelihood that an infected animal will be in contact with an uninfected animal for long enough to pass on the virus and facilitate animal-to-animal transmission. Furthermore, stress can have a negative impact on the ability of animals to raise a robust immune response to infection. Influenza viruses are inactivated by exposure to the ultraviolet rays in sunshine, therefore the virus may survive for longer in the environment indoors. Furthermore, unless there is an extremely efficient ventilation system, there will be a greater accumulation of virus in the environment. During the outbreak of HPAI virus in Canada in 2004, testing of air samples taken inside the infected poultry houses confirmed high levels of virus and some virus was also found in air sampled from outside the barns (Power 2005). During the 2003 epidemic of HPAI among poultry in the Netherlands, movement bans and other control measures were imposed soon after the virus was detected, yet additional premises became infected. It was therefore necessary to implement culling of contiguous flocks. In all, 255 flocks were affected and around 30 million birds culled[17]. The route of spread was not identified 80–90% of the time but the likelihood of a farm becoming infected was higher if the farm was near a farm that had just become infected. The continued farm-to-farm transmission despite the control measures put in place illustrates the difficulty of controlling an outbreak of HPAI where there is a high density of poultry-rearing units.

A review of studies conducted in various studies that had analysed the factors influencing the occurrence of H5N1 HPAI outbreaks found three associations that held true across several of the studies[18]. These were: the presence of ducks or other domestic waterfowl at the location, several human factors such as population density and distance to roads and the presence of water such as a pond, stream or flooded land. There was a paucity of studies in Egypt and Indonesia and not all studies analysed considered the same set of factors. Individual studies in Thailand and Nigeria found that H5N1 HPAI outbreaks were significantly more likely to occur where poultry were purchased from another farm[19]. The Nigerian study also found a positive association with farms receiving visitors and workers living off the premises. Finally, a study in Vietnam found that flocks with a higher density of birds were more likely to be affected[20].

Infection of ducks is an unusual feature of the on-going H5N1 HPAI outbreak, although ducks were not directly implicated in the early spread of the virus in Hong Kong[21]. Fatal infection of ducks with H5N1 HPAI was first reported in 2002-03 in Hong Kong[22]. It has been demonstrated that ducks shed the virus in their faeces and *via* the respiratory route for at least 17 days[23]. Hulse-Post *et al*. (2005) also showed that during this extended infection, the virus lost its pathogenicity, meaning that ducks could act as 'silent' carriers of the H5N1 virus which, if transmitted to terrestrial poultry, could re-emerge in a highly pathogenic form. In Thailand, placing restrictions on grazing of ducks and killing all ducks in flocks where infection was detected reduced infection in ducks with a concomitant reduction of cases in poultry[24]. This does not prove a causal link, but suggests that ducks may play an important role in the transmission of virus to other species.

Preventive measures

There are numerous biosecurity procedures that can be adopted to minimise the risk of spread of influenza. Ideally, poultry being introduced onto a farm should be quarantined from other animals for a period to minimise the risk of bringing influenza into the facility. There should be procedures for disinfection when moving equipment and for people moving between buildings and premises. Buildings where poultry are housed should have screens or nets in place to limit the entry of wild birds. The use of lagoons and ponds on poultry units by waterfowl should be discouraged and water from such sources should not be used for washing down poultry houses etc. without treatment to destroy any potential contaminating viruses[25]. Due to the potential for ducks to act as a reservoir of infection, separation of domestic waterfowl from terrestrial poultry should be maintained wherever possible, but particularly in live bird markets and larger commercial units[26].

It is often assumed that large commercial units are more likely to institute stringent disease prevention measures, in part because of the greater risks of disease spread associated with intensive farming. However, studies have called this assumption into question with failure to provide simple biosecurity measures such as disinfection footbaths or protective clothing for poultry workers on commercial farms[27] and violation of biosecurity protocols for movement of animals and people[28]. A thorough analysis of data from Thailand suggested that commercial poultry production is not associated with any reduction in risk of H5N1 HPAI occurring compared with backyard farms[29].

Heightened surveillance of people working with poultry could enable early detection of an emerging potential pandemic virus in future. Vaccination of people working in intensive poultry units (including veterinarians) against a potential pandemic influenza (e.g. H5N1), preferably with a killed virus vaccine has been proposed[30]. However, it is difficult to predict exactly what will be the next pandemic virus and vaccinating a farm worker with a strain that only provides partial immunity could lead to infection without any clinical signs. This enhances the risk that they continue with their daily lives and pass on the infection to susceptible contacts. Vaccinating farm workers against regular seasonal human influenza would minimise the risk of them becoming dually infected with avian and human influenza strains, which could give rise to generation of a reassortant, and may provide some cross-immunity against people being infected with avian influenza viruses. Farm workers with flu-like symptoms should be advised to stay away from work until their symptoms have cleared; this rule may not be adhered to by workers if missing work entails a penalty such as deduction of wages.

Flocks can be vaccinated against influenza, and increasingly this is the case in some areas[31]. However, as for vaccination of people, use of an imperfect vaccine may mean that infection occurs without clinical signs, leading to unseen transmission. It is therefore important that the use of vaccines does not lead to complacency with respect to routine biosecurity measures. In 1993, and H5N2 LPAI virus emerged in poultry in Mexico and HPAI strains subsequently appeared in 1994-1995[32]. Although no HPAI virus was reported since 1996, LPAI virus continued to circulate raising concerns that an HPAI strain could re-emerge therefore, a widespread programme of vaccinating poultry was introduced. This represented the first time long-term vaccination of poultry had been used, thus providing the opportunity to examine the potential consequences for viral evolution. Genetic analysis revealed that the Mexican H5N2 viruses were evolving more rapidly than similar viruses in areas where no vaccines were used. It was suggested that this increased rate of antigenic drift was driven by vaccination. It was also shown that the drifted viruses were sufficiently different from the vaccine virus strain that vaccine-induced antibodies no longer protected birds against infection[33]. It is therefore also important to adequately monitor vaccination programmes and to update vaccine strains as necessary.

There are two groups of antiviral drugs (the adamantanes and neuraminidase inhibitors) available for treatment or prophylaxis of influenza in people. These are not licensed for use in birds, but a joint statement from the FAO (Food and Agriculture Organization of the United Nations), OIE (World Organization for Animal Health) and World Health Organization expressed concern that some of these drugs are being mis-used in poultry, as a result of which drug-resistant strains of H5N1 HPAI are emerging such that the drugs will become ineffective for treatment of human infections with the H5N1 virus.

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