Some Epidemiological Aspects of Avian Influenza
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Summary
Introduction of avian influenza (AI) viruses into poultry flocks usually originates from wild water fowl or live bird markets that are endemically infected with low pathogenicity avian influenza (LPAI) virus. Next if the virus is of the H5 or H7 serotype, it may mutate into a high pathogenicity avian influenza (HPAI) strain. Consequently, primary introductions of AI viruses into commercial poultry flocks should be prevented by bio-security measures. Moreover, surveillance for antibodies or clinical signs of LPAI may detect virus infection in commercial poultry before mutation to HPAI has taken place. During an epidemic, the spread of AI virus between flocks is mainly caused by the transfer of infected birds or faeces attached to persons, equipment or vehicles. Thus movement of personnel and equipment must be strictly controlled and strict hygiene enforced. In addition, during epidemics of HPAI, infected flocks have to be stamped out. However, this may not be sufficient to stop epidemics in densely populated areas. In such areas either very dramatic measures such as pre-emptive culling of contiguous flocks, depopulation of centre areas have to be done to stop an epidemic or emergency vaccination should be applied.

Introduction
Avian influenza viruses are highly infectious micro-organisms that primarily affect birds. Nevertheless, they have also been isolated from a number of mammals, including humans. Avian influenza virus can cause large economic losses to the poultry industry because of its high mortality. Although there are pathogenic variants with a low virulence which generally cause only mild, if any, clinical symptoms, the subtypes H5 and H7 that can mutate from a low to a highly virulent virus and should be taken into consideration in eradication strategies (Landman and Schrier, 2004).

Avian influenza (AI) is caused by Influenza A viruses, which are common pathogens in various animal species, such as pigs, birds, horses and humans (Alexander and Brown, 2000). Virus strains of high pathogenicity (HP) of AI (HPAI) can be devastating to poultry flocks because of their high transmission rate and high associated mortality rate (Alexander, 2000). HPAI is, therefore, categorized by the Office International of Epizootics (OIE) as a member of list A diseases.

In 2004, South-East Asia was struck by a huge epidemic of AI. In that epidemic million of domestic birds died or had been scarified, as well
as several humans died due to infection by the A.I. virus. Devastating epidemics have been reported from USA, Pakistan, Mexico, Italy, Hong Kong and the Netherlands (Swayne and Halvorson, 2003). Also, in the last two countries, fatal human cases of HPAI virus infection have been reported (Mounts et al., 1999; Fouchier et al., 2004).

**Diagnosis of Avian influenza**

Currently, virus isolation (VI) in embryonating chicken eggs and subsequent HA and neuraminidase subtyping by serological methods constitute the standard methods for AIV detection and subtype identification. Although VI in embryonating eggs is a sensitive method, it may take 1 to 2 weeks to obtain results, by which time the results may no longer be relevant. Conversely real-time reverse transcriptase PCR (RRT-PCR) can be a rapid assay; results, including subtyping, may be available in less than 1 day. It can also be less expensive on a cost-per-sample basis than VI in embryonating eggs.

Standard RT-PCR has been previously applied to the detection of avian influenza virus (Lee et al., 2001, Starick et al., 2000; Suarez, 1997) and each of the 15 HA subtypes (Lee et al., 2001). Additionally, an RRT-PCR assay for influenza virus has been developed; however, it is a two-step, multiplex RT-PCR assay based on human influenza virus sequences for the detection of influenza virus types A and B (Van Elden et al., 2001). One-step RT-PCR with hydrolysis probes, as described by Holland et al (1991) and Livak et al., (1995) has been successfully applied to the detection of various RNA viruses (Martell et al., 1999). RRT-PCR offers the advantages of speed and no post-PCR sample handling, thus reducing the chance for cross-contamination versus standard RRT-PCR. The high sensitivity, rapidity, reproducibility and specificity of the AIV RRT-PCR can make this method suitable for diagnosis and for the evaluation of viral load in field specimens (Livak et al., 2006).

To control future epidemics of HPAI, knowledge of the effectiveness of the control measures implemented during an epidemic is important. The epidemiology of AI viruses will be briefly described because this knowledge is essential for understanding the rational behind control measures. In this paper a short overview is given of the measures to control AI, and these include measures to prevent the primary and secondary introduction of AI viruses into poultry flocks.

**Epidemiology of Avian Influenza**

Primary introduction of AI viruses into commercial poultry flocks originates from waterfowls and live bird markets. The relative risk associated with each of these sources varies depending on the likelihood of direct or indirect contacts with susceptible poultry (Stegeman et al., 2004).
Free flying aquatic birds, especially of orders *Anseriformes* (ducks and geese) and *Charadriiformes* (shorebirds, gulls, tems and auks) serve as an important reservoir of low pathogenicity (LP) AI virus strains (Alexander, 2000). These LPAI virus strains can be transmitted from waterfowl to poultry by infected faeces either through direct contact or indirectly through contamination of feed, water, or free-range area. Next, if the LPAI strain is of H5 or H7 subtypes, the virus may mutate into a HPAI strain. In addition to waterfowl, the bird markets system poses a significant risk to the introduction of AI viruses into poultry flocks (Swayne and Halverson, 2003). Because of the continuous marketing system process, LPAI virus can persistently circulate in these systems. Indirect contacts through contamination of persons, equipment or vehicles could be transmitting the virus to industrial poultry flocks. Additionally, companion or pet birds may also serve as a source of infection, because AI viruses have been recovered from caged birds, usually during quarantine. However, transmission from these sources to poultry has not been documented (Stegeman et al., 2004).

In case of secondary transmission, birds excrete AI viruses from both the respiratory and digestive tract. Thus, within a poultry house, bird-to-bird transmission is probably possible by aerosol and ingestion. Between flocks, infected poultry faeces appear to be a most likely source of transmission, man-associated contacts being the main route (Swayne and Halvorson 2003). In several specific accounts strong evidence has implicated the movement of caretaker, farm owners and equipment, trucks and drivers moving birds or delivering food, and artificial insemination in the spread of the virus. Birds or other animals which are not themselves susceptible to infection may also become contaminated and transmit the virus. Moreover, shared water or food could become contaminated and serve as an infection source. Finally, there is some evidence that windborne spread may have played a role amongst very closely situated farms and that flying insects could become contaminated with faeces (Swayne and Halvorson, 2003). Vertical transmission of AI viruses is unlikely, because AI are embryo lethal (Stegeman et al., 2004).

**Control of Avian Influenza**

The basic means for prevention of primary introduction of influenza viruses into poultry flocks is to minimize the direct and indirect contacts between birds in these flocks on the one side and wild water fowl, live bird markets and pet birds on other side. Consequently, bio-security is the way to minimize the risk of the introduction of AI viruses into commercial poultry flocks. The most important bio-security measures are poultry kept indoors, only one species of poultry on the premises, no pet birds on the same premises as commercial poultry, all in-all out production system (reduces the risk of an endemic infection with LPAI
viruses), no items that attract wild birds to premises (such as ponds), feed and drinking water for poultry are not accessible to wild birds, poultry workers do not have access to other birds, fence around the poultry house, only those persons that really need to be there are admitted to the poultry house and persons change clothes and boots before entering the poultry house.

In addition to preventing the introduction of AI viruses it is desirable to conduct surveillance for antibodies or clinical signs of LPAI. Such a programme may detect LPAI virus infection in commercial poultry before mutation to HPAI has taken place. When adequate measures are taken in such a situation, an epidemic of HPAI may be prevented (Stegeman et al, 2004).

The basic factors that determine secondary transmission of AI are the amount of virus produced by an infected flock (infectivity), the amount necessary to infect a susceptible animal (susceptibility), the amount of virus that is transferred during a contact, the contact rate, and the number of contacts between flocks. Consequently, interventions reduce transmission through their effect on one or more of these factors (Koopman and Longini, 1994; Stegeman et al, 2004).

The amount of virus produced by an infected flock can be reduced by the killing and removal of these flocks. This approach are followed in most countries, during the epidemics in Italy 1999-2000, the Netherlands 2003, most countries in Asia and the US 2003-2004. The culling of infected flocks is often accompanied by depopulation (pre-emptive culling) of contiguous flocks. Some of these contiguous flocks may have already been infected and, consequently, will be depopulated when their infectivity is still low. Despite their rigorous nature, it is still questionable whether these measures are sufficient to stop an epidemic in poultry dense areas and in areas with many turkeys (Swayne and Halvorson, 2003). In such areas virus circulation may remain until all poultry have been depopulated.

Another way to reduce the amount of virus produced by an infected flock is vaccination. Vaccination reduces the amount of virus necessary to infect a susceptible host animal. Experimental studies have demonstrated that inactivated mono-valent and polyvalent virus vaccines with adjuvant are capable of inducing antibodies and providing protection against mortality, morbidity and decline of egg production. Moreover, vaccination significantly reduces the excretion of virus (Boyle et al, 2000; Swayne et al, 2001; Tollis and DiTrani, 2002; DiTrane et al, 2003), which may reduce virus spread in an infected area. However, in several countries, vaccines designed to contain or prevent HPAI are especially banned or discouraged by government agencies, because they may interfere with stamping out control policies. It is argued that, since
immunized birds are still liable to infection and excretion of virus, the danger is not contained. In addition, since immunized birds may not show clinical signs, infection may go unnoticed for long periods and even exacerbate the spread of the virus. However, most HPAI control regulations reserve the right to vaccinate, because stamping out policy may be unsuccessful, unethical and depending on, economically the desirable situation. Because of the rare nature of HPAI, pre-emptive prophylactic vaccination on a large scale is not desirable. However, vaccination was applied to establish a buffer zone around an outbreak. It is important that the use of vaccination must be regarded as complement to strict bio-security, quarantine and other measures aimed at preventing the virus spread.

To reduce the amount of virus that is transferred during contact, persons must change boots and clothing upon entering the premises. Moreover, equipment that come into direct contact with poultry or their faeces should not be moved from farm to farm without adequate cleaning and disinfection. Cleaning of faecal material and disinfection of egg shells may be necessary to prevent the hatchery-associated dissemination of AI viruses (Swayne and Halvorson, 2003). In addition, the contact rate between flocks and the number of flocks that contact with each other should be minimized by measures such as a ban on transport of poultry products, strict control of the movement of personnel and equipment. Furthermore, it is important to keep the traffic area, near the poultry house, free from becoming contaminated with manure.

Moreover, a growing number of human cases of avian influenza, some of which are fatal, have paralleled the outbreaks in commercial poultry. There is great concern about the possibility that a new virus subtype with pandemic potential could emerge from these outbreaks. From a human health perspective, it is essential to eradicate the virus from poultry; however, the large number of small-holdings with poultry, the lack of control experience and resources, and the international scale of transmission and infection make rapid control and long-term prevention of recurrence extremely difficult. In the Western world, the renewed interest in free-range housing carries a threat for future outbreaks (Landman and Schrier, 2004)

To prevent new epidemics the poultry industry should focus on minimizing contacts between commercial poultry flocks and waterfowls, live bird markets and pet birds. Moreover, to stop future epidemics, movement of personnel and equipment must be strictly controlled and strict hygiene enforced. In addition, infected flocks need to be stamped out and contiguous flocks must be pre-emptively depopulated. The latter may, however, be insufficient in densely populated area, leading to a choice to either depopulated an entire area, or vaccinate all poultry in a
buffer zone. In all cases it is essential to educate poultry workers in how viruses are introduced, spread, and how such condition may be prevented (Stegeman et al., 2004).

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