Outdoor ranging of poultry: a major risk factor for the introduction and development of High-Pathogenicity Avian Influenza

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Abstract

High-Pathogenicity Avian Influenza (HPAI) is an extremely infectious viral disease of poultry. Public health concerns were raised when six persons died in Hong Kong in 1997 after exposure to HPAI-infected poultry. Its danger became imminent in the recent HPAI epidemic in South-East Asia when the virus expanded its geographical range via parts of central Asia to Europe, Africa and the Middle East. Wild birds are frequently carriers of influenza A viruses. Nearly all Avian Influenza (AI) viruses isolated from wild birds are low-pathogenic and cause no clinical problems in these birds. Only after low-pathogenicity viruses are introduced in poultry, in particular in chickens and turkeys, high-pathogenicity mutants emerge after a variable length of time. Biosecurity is the first line of defence against an introduction of AI into commercial poultry flocks. Any conceivable contact between possibly contaminated animals, areas around poultry houses contaminated with faecal material from wild birds and contaminated abiotic vectors on the one hand and domestic poultry on the other must be avoided. In this paper we shall discuss the worldwide occurrence of HPAI outbreaks, the existence of AI virus infections in wild birds, and possible strategies to reduce the risk of the introduction of AI viruses into domestic poultry flocks, with special reference to free ranging.

Additional keywords: wild birds, public health risk, Avian Influenza ecology

Introduction

Avian Influenza (AI) is a viral disease of birds caused by influenza A viruses. Influenza A viruses that infect poultry can be divided into two groups: low-pathogenicity (LPAI) and high-pathogenicity avian influenza (HPAI) viruses on the basis of severity of the disease following experimental infection of chickens (Alexander, 2002). Waterfowl and shorebirds (wild and domestic) form the major natural reservoir and
source of all known influenza A viruses. The virus particles carry two glycoproteins on their surface: haemagglutinin (HA) and neuraminidase (NA). Influenza A virus can be divided into subtypes based on the possession of one of the 16 distinct haemagglutinin antigens (H1–H16). Each of the haemagglutininine types combines with one of 9 neuraminidase antigen types (N1–N9). Virtually all combinations of HA and NA subtypes have been isolated from wild bird species (Webster et al., 1992). The HPAI viruses that cause major diseases in poultry belong to the H5 and H7 subtypes, although not all viruses of these subtypes are HPAI viruses. The influenza A viruses of the remaining subtypes belong to the group of LPAI viruses.

High-Pathogenicity Avian Influenza is an extremely infectious and fatal poultry disease. Clinical signs associated with HPAI viral infections can vary considerably and depend on bird species, age, sex, concurrent infections, virus strain and environmental conditions (Swayne & Halvorson, 2003). Chickens and turkeys infected with HPAI virus are mostly found dead (up to 100% flock mortality within only a few days) with only a few clinical signs like general depression, apathy, reduction in normal vocalization, decreased feed and water consumption, swollen head and wattles, diarrhea, and haemorrhage with cyanosis of the skin, particularly visible on wattles, combs and legs. These symptoms are usually seen in birds that take some time to die. Tremors of the head, paralysis of the wings, abnormal gait, and lack of co-ordination are often seen in turkeys (Swayne & Suarez, 2000; Capua & Mutinelli, 2001; Swayne & Halvorson, 2003). However, most AI virus strains are of low pathogenicity and typically cause mild respiratory problems or a decrease in egg production and/or water and feed intake.

HPAI infections of domestic bird species like ducks and geese usually do not cause severe disease. However, clinical signs and mortality in these species have been observed in Italy and recently in Asia. In ostriches and quails the mortality rates vary, but generally do not reach 100%. For these reasons there is an increased risk of late diagnosis or misdiagnosis in birds other than chickens and turkeys, leading to delayed notification.

Wild bird surveys performed in North America showed that particularly Anseriformes (like ducks and geese) and shorebirds are frequently carriers of influenza A viruses. Recent surveys in Europe confirmed the higher prevalence of LPAI viruses in ducks compared with other waterfowl but no viruses were isolated from shorebirds, indicating regional differences (Fouchier et al., 2003a). Nearly all viruses isolated from wild birds are low-pathogenic, and the few that were highly pathogenic could be associated with major outbreaks in domestic poultry. Only after LPAI viruses are introduced in poultry, particularly in chickens and turkeys, HPAI mutants emerge after a variable length of time (Alexander, 2003). In 19 of the 24 outbreaks that have been reported during the past 46 years it was shown that the virus was introduced from wild fowl and then mutated into an HPAI variant, either in a short period (15 cases) or after several months (4 cases).

In this paper we shall discuss the occurrence of worldwide HPAI outbreaks, the existence of AI virus infections in wild birds, and possible strategies to reduce the risk of introduction of AI viruses into domestic poultry flocks, with special reference to free ranging.
HPAI outbreaks in domestic poultry worldwide

Pathogenicity is the result of interaction between host and virus. An influenza virus that is pathogenic for one avian species will not necessarily be pathogenic for another avian species. The pathogenicity of an influenza virus is determined by more than one gene. Haemagglutinin (HA) is an important trait of pathogenicity because it binds to the cellular receptor and thus largely determines tissue tropism. Mutations in the receptor-binding site of the viral HA can alter the ability of viruses to infect different hosts (Naeve et al., 1984). Another important feature is the cleavability of HA (Bosch et al., 1981). Haemagglutinin is produced as a precursor protein that has to be cleaved for the virus to become infectious. Normally, in the respiratory and the intestinal tract, HA is cleaved by trypsin-like enzymes that are present in the lumen of both tracts. However, HPAI viruses possess HAs that are readily cleaved by ubiquitous, intracellular proteases that are present in a variety of cells, allowing infectious virus to be produced by many cells throughout the body. Therefore, HPAI viruses spread throughout the bird, damaging vital organs and tissues, which results in disease and death (Roth, 1992). The difference between HA of LPAI and HPAI viruses is that the latter have a series of basic amino acids at the cleavage site, whereas LPAI viruses have a single basic amino acid at that site.

On several occasions in the past, e.g. in the USA (Bean et al., 1985), Mexico (Garcia et al., 1996), Italy (Capua & Marangon, 2000), Chile (Rojas et al., 2002), the Netherlands (Elbers et al., 2004) and Canada (Bowes et al., 2004), it has been shown that an HPAI virus strain developed from an LPAI virus strain through mutations mostly involving nucleotide insertions near the cleavage site. Mutations of influenza viruses are assumed to occur randomly and are attributed to mistakes made by the polymerase needed for virus genome replication. Mutants will survive and emerge whenever they have a selective growth advantage over the majority of the virus population. A positive selective pressure only seems to exist in galliform birds. Therefore, the longer the presence and the larger the spread of LPAI H5 and H7 viruses in poultry the more likely HPAI virus will emerge (Alexander, 2003). So mutation, which is a stochastic event, combined with mutant selection explains the variability in time before the emergence of HPAI from an LPAI virus.

In the last 40 years of the 20th century, reports on severe HPAI outbreaks have, fortunately, been infrequent (Table 1). In the last 5 years, however, increased occurrence of HPAI is noticed, especially in South-East Asia, where the disease seems to have become endemic and its eradication has not been achieved so far, allowing its spread to other continents both by trade and by migratory birds.

Influenza A viruses in wild birds

Infection pathways

In spite of differences between regions in success rate of virus recovery from wild birds, influenza viruses are predominantly isolated from ducks, geese, shorebirds and
gulls, constituting the reservoir of influenza A viruses in nature (Stalknecht et al., 1998; Fouchier et al., 2003a, b). AI viruses preferentially infect cells lining the intestinal tract of waterfowl. As a result, waterfowl shed enormous amounts of virus with their faeces for long periods, leading to heavily contaminated lake and pond water in their habitat (Hinshaw et al., 1979). This contrasts sharply with influenza infections in mammals and even in other avian species where viral shedding more or less stops after seroconversion. Intestinal infection in ducks is common, yet harmless and can involve at the same time a multitude of different influenza A viruses. The non-virulent nature of the intestinal infection in ducks may be the result of virus adaptation to the host over many centuries, creating a reservoir that perpetuates the virus without endangering the host (Hinshaw, 1986).

A survey of migratory waterfowl in North America indicated that up to 60% of the juvenile birds may be infected as they congregate in marshalling areas prior to migration (Hinshaw et al., 1985). Furthermore, none of the ducks examined showed any clinical symptom of infection. In the remaining periods the success rate of virus recovery from samples of migratory ducks dropped. In Northern Europe the overall success rate of virus detection was approximately 20% in samples collected

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¹ In case of widespread outbreaks affecting more than one species, the isolate from the first outbreak identified is listed.

² Cambodia, China, Indonesia, Japan, Lao PDR, Malaysia, Republic of South Korea, Peoples Republic of North Korea, Thailand and Vietnam reported the disease in this year. So far the relationship of these viruses to A/Hong Kong/97 (H5N1) remains unclear.

³ This virus did not kill chickens infected experimentally, but had multiple basic amino acids at the HAO cleavage.
from migratory ducks in the autumn of 2002. Fourteen of the 16 HA subtypes were detected in 200 positive samples of which 12 were of the H7N7 and 16 of the H5N2 subtype. In a study in northern Italy, which was still ongoing at the time of writing, 9 H7 viruses have been isolated from domestic waterfowl and game birds between 2003 and the beginning of 2005. Seven of these were isolated from wild birds (mallards and teals) and two from domestic waterfowl in backyard flocks (Terregino et al., 2005).

**Persistence and mutation of influenza viruses**

It is not clear how influenza viruses are maintained from year to year, but there is evidence that the viruses are maintained in duck populations or survive in their habitat. Ducks have been shown to excrete virus for as long as 30 days, so it would not require many virus passages to maintain the viruses in the population (Hinshaw, 1986). It is possible that the viruses are maintained – even at a low level – in the wild duck population by transmission to susceptible birds throughout the year until the next breeding season results in a new group of susceptible juveniles. Like ambient air temperature, the ambient surface water temperature declines in autumn and winter in the northern hemisphere, and low temperatures prolong the survival of AI virus. A single duck may excrete as many as 10 billion 50% egg-infective doses of influenza virus in a 24-hour period (Webster et al., 1978). As a result, a few migratory ducks might carry the virus and infect stationary ducks that replicate and excrete influenza virus into the water over longer periods, so that the water of lakes, marshes and ponds could become increasingly infectious (Halvorson et al., 1985).

In the past many surveys in North America, and more recently also in Europe have shown that H1, H2, H3, H4, H6, and H11 subtypes predominated in AI virus isolations from ducks, and that the H5 and H7 subtypes only occurred incidentally (Lang, 1981; Halvorson et al., 1983; 1985; Hinshaw et al., 1986; Nettles et al., 1986; Alfonso et al., 1995; De Marco et al., 2003; Fouchier et al., 2003a, b; Hanson et al., 2003). Recently there have been indications that the H5N1 virus, which was the source of the 1997 HPAI epidemic in Hong Kong, has persisted in ducks in South-East Asia over the last years, and has become progressively more pathogenic for mammals (Chen et al., 2004). It is suggested that birds like gulls and shorebirds, which carry subtypes different from those isolated from ducks, provide an additional influenza gene pool enabling influenza to evolve (Kowaoka et al., 1988). The genetic information of influenza viruses is divided over eight segments that are randomly packed during the formation of a virus particle. Consequently, whenever two different viruses come together in the same cell of an infected animal many different combinations of the gene segments can arise. In all, 256 combinations are possible but not all are viable. This process is called re-assortment, and viable viruses that emerge are called re-assortants. Since many different bird species carrying different influenza subtypes share habitats such events can occur in nature. The Asian H5N1 virus evolved after different re-assortment events over several years. Which subtypes donated gene segments to the H5N1 virus remains largely unknown.
Transmission of the influenza virus from wild birds to poultry

There is ample evidence that influenza viruses of wild birds, in particular wild waterfowl, were transmitted to poultry. Wild waterfowl was considered a significant source of viruses for free-range turkeys and this has been mentioned as an important route in areas like Minnesota and Wisconsin, which are located along a major flyway. It was established that the outbreaks in turkeys coincided with the presence of migratory ducks (Halvorson et al., 1983; 1985; Hinshaw et al., 1986). Between 1978 and 2000, poultry farmers in Minnesota experienced 108 introductions of LPAI viruses of various HA and NA subtypes from migratory ducks into turkeys (Halvorson, 2002). These Minnesota cases resulted from close direct contact between seasonal migratory juvenile ducks (September to November) and free-range turkeys, or the use of AI-virus-contaminated lake or pond water for turkeys reared indoors. Although free-range and semi-confinement rearing have represented historically less than 5% of turkey production, this minor production method has been the introduction point for LPAI viruses into commercial turkeys in Minnesota with disastrous results. With the H5N1 HPAI outbreak in poultry and the human infections in Hong Kong in 1997, the Minnesota production companies agreed to stop free-range rearing of turkeys to eliminate the introduction of LPAI viruses from waterfowl and prevent a potential public relations problem should an outbreak of LPAI or HPAI occur. As a result, during the period 1997–2000 only 28 flocks became infected with LPAI viruses, mostly from swine H1N1 influenza A virus.

California serves mainly as a wintering site rather than a breeding ground in the waterfowl life cycle. Mainly ducks and geese, coming from the north via the Pacific flyway, stay in the wetlands of the Central Valley where food is plentiful. Birds start to arrive in August and immigration ends around 1 January. Northern migration starts in late January with the heaviest flow in February. Although no direct link was ever established between viruses in wild waterfowl and poultry, all 12 influenza virus isolates (mainly H6 subtypes) were made in the period September–April (McCapes et al., 1987).

Another example is the outbreak in Chile in 2002 (Rojas & Capua, 2002) on a poultry farm managed at a high biosecurity level. The outbreak was caused most likely by using drinking water from a pond on the premises that was frequented by wild birds, demonstrating how easily breaches in biosecurity barriers can be overlooked. In the beginning the symptoms were a low mortality rate, a slight drop in egg production, and peritonitis. An LPAI H7N3 virus was isolated from birds in some of the houses of the broiler breeder farm. However, within 3 weeks the mortality rate increased dramatically and HPAI H7N3 was isolated from dead birds. This illustrates that even when no free-range facilities are being used, biosecurity is as good as its weakest link.

Small flocks of domestic waterfowl (ducks and geese) raised outdoors could also constitute a possible route of introduction, particularly if they are mixed with other species of domestic poultry and are reared under common management. Opportunity for AI virus introduction is provided by the tendency of domestic ducks to attract wild ducks. Such introductions can remain unnoticed because infection of ducks even with HPAI mostly does not involve disease. The partial depopulation and restocking often
practised on this kind of premises will enable the maintenance and selective adaptation of influenza viruses. In addition, trading and exchanging of live birds may be responsible for the perpetuation of infection and the spread to other farms. Furthermore, transmission of AI virus is readily accomplished by virtually anything contaminated with faecal material, e.g. feed, water, equipment, supplies, cages, clothes, delivery vehicles, professional persons and insects (Swayne & Halvorson, 2003).

**Phylogenetic studies**

Generally, the presumed transmission route from wild birds to poultry is supported by phylogenetic studies. High-Pathogenicity Avian Influenza and LPAI H7 subtype viruses do not constitute a separate phylogenetic lineage or lineages. Phylogenetic studies indicate that HPAI arises from non-pathogenic strains (Rohm et al., 1995; Banks et al., 2000) by *in vitro* selection of mutants virulent for chickens from an non-virulent H7 virus (Li et al., 1990). It appears that such mutations occur only after the viruses have moved from their natural wild bird host to poultry. However, as explained above, the mutation to virulence is unpredictable and may occur very soon after its introduction to poultry, as in the case of outbreaks 1–4, 6, 8–12, 14, 15, 17, 19, 20 and 22 in Table 1, or after the LPAI virus has circulated for several months, as in the case of outbreaks 7, 13, 16 and 18. The viruses responsible for the Chilean (19) and the Canadian outbreak (22) apparently arose as the result of mutation by a mechanism different from the one playing a role with other HPAI viruses. From studies it became clear that the 11 amino acid insertion occurred by recombination, introducing a section of the NP gene (Suarez et al., 2004) and a 7 amino acid insert of the matrix gene into the HA gene (Pasick et al., 2005).

The Dutch outbreak that started at a free-range farm was caused by an H7N7 virus (Elbers et al., 2004). This virus is believed to be a re-assortant of an H7N3 and an H10N7 virus that were isolated from mallards in 2000 during survey studies of migratory wild birds in the Netherlands (Fouchier et al., 2004). Sequence analyses showed that, apart from the cleavage site, the H7 haemagglutinins had only two and the N7 neuraminidases eight amino acid differences. The H7N7 virus had probably been circulating for some time in wild ducks as an LPAI virus before it was introduced in one of the two houses of the free-range farm. Shortly after its introduction, an HPAI variant of the LPAI H7N7 virus emerged in this house. The variant was transmitted to the second house causing high mortality among its fully susceptible chickens. This view is supported by the absence of a high mortality rate and positive serology in the first house, whereas high mortality and negative serology were observed in the second one. Typically, LPAI virus will cause little disease and therefore induce immunity, protecting the birds against HPAI whereas birds initially infected by HPAI virus will already have died before the onset of immunity.

A cross-sectional serological survey of the Dutch poultry population was carried out in the second week of March 2003 to investigate whether LPAI viruses had been circulating prior to the emergence of the HPAI virus. The serological screening of 28,018 serum samples from 1193 randomly selected poultry farms located outside the survey areas, showed that LPAI H7 virus infections had occurred on three neigh-
bouring farms: two free-range poultry farms and one turkey farm, all located in the southwest of the Netherlands. No antibodies against the neuraminidase N7 subtype were detected in the serums from these farms. LPAI H7N3 virus was isolated from dead turkeys that had been stored in a freezer since December 2002, following clinical problems at the end of that year. The result most likely points to an introduction of the H7N3 mallard virus some time in the autumn of 2002, separate from the H7N7 epizootic (De Wit et al., 2004). In the AI serological monitoring programme that started in 2004, antibodies to H7 were detected on a free-range farm in the municipality of Uithuizermeede, located in the northern part of the Netherlands near the Dutch Shallows and the Dollard, both harbouring important duck populations. The antibodies probably are the result of LPAI H7 virus because a retrospective study of flock performance only revealed a drop in egg production 6 weeks before blood samples were collected.

Sequence analysis of viruses isolated in the ongoing Italian wild bird survey showed that the H7 gene showed a 99.3% homology at the nucleotide level between the isolates from the backyard flocks and the isolates obtained from wild birds (Terregino et al., 2005). These preliminary data may support the theory that backyard poultry represents one of the links that connect the AI in wild bird populations to poultry. For this reason it is imperative that the next transmission step (from backyard to intensively reared poultry) is avoided. This can be achieved through the implementation of rigorous biosecurity measures.

Prevention of exposure

Biosecurity

Biosecurity is the first line of defence against an introduction of AI and probably the only defence as long as preventive/prophylactic vaccination of flocks at risk is excluded. Biosecurity can be very effective, as is demonstrated by specific pathogen-free chickens mainly reared for veterinary vaccine production and diagnostic purposes. Such animals are raised in positive pressure houses and filtered air. Unfortunately these housing systems are expensive and therefore not economically feasible for intensive poultry production.

Biosecurity comprises two elements: bioexclusion and biocontainment. Biocontainment means preventing the virus from spreading from infected premises. Bioexclusion refers to measures that exclude the introduction of infectious agents to non-infected premises. Obviously, there is considerable overlap between both concepts since good biocontainment practices will reduce the number of new introductions.

Good bioexclusion and biocontainment depend on the formation of a barrier between farms and the outside environment. This sounds simple but in practice can be difficult to implement successfully. Many items and people routinely enter poultry farms, including replacement birds, feed, water, farm workers, consultants, veterinarians, poultry buyers, catchers and vaccination crews. In addition, it is impossible to completely prevent the access of vermin to poultry houses.
Bioexclusion
Obviously, bioexclusion is particularly difficult to achieve in free-range farming, because of the free access of wild birds secreting virus with their faeces or passively carrying virus from nearby infected ponds. Also the use of open water sources for drinking purposes may introduce the virus from wild birds into domestic poultry.

Poultry farmer organizations in the Netherlands are suggesting to keep poultry inside during the wild-bird migration period in spring (Anon., 2005). However, the success rate of virus isolations of samples collected from wild birds in the Netherlands is highest between September and January, suggesting that the risk is higher in this period than in spring (R.A.M. Fouchier, pers. communication).

To achieve bioexclusion of AI, any conceivable contact between possibly contaminated animals, contaminated areas around poultry houses and contaminated abiotic vectors on the one hand and domestic poultry on the other must be avoided. For free-range poultry farms this can be accomplished by:
1. Double fencing and roofing (‘Wintergarten’) of the free-range area, excluding access of possibly infected wild birds and other animals to the free-range area, and preventing possible contamination of the area by faecal material from infected wild birds and other animals.
2. Landscape gardening that discourages visits of ducks and geese. Ducks and geese alight in open field whereas chickens and turkeys favour to grub in bushes.

In Minnesota (USA), the Minnesota Cooperative AI Control Program states the following preventive measures that are part of bioexclusion (Halvorson, 1987):
1. Do not hunt, trap or fish on the same day you take care of poultry; bird hunters should be aware that the game they bag is likely to be infected;
2. Do not allow clothes used for hunting, trapping or fishing on poultry farms unless they have been laundered;
3. Do not allow vehicles, boats, or equipment used for hunting, trapping or fishing to enter a poultry farm unless they have been washed with detergent and disinfected;
4. Do not bring game or fish onto a poultry farm unless it has been dressed and packaged;
5. Isolate ponds, sloughs and streams from poultry; do not walk directly from such environments into poultry houses; do not use pond water for watering poultry;
6. Do not allow pet animals, like cats, dogs, rabbits etc, to enter a poultry house, pen or range;
7. Have a control program for wild birds and rodents; trapping of these animals must occur away from poultry (outside poultry house) and should preferably be done by someone other than poultry farmer or farm help; biosecurity measures should be in place to keep those animals outside the poultry house.
8. No other birds (poultry of any kind, especially domestic waterfowl) should be allowed on the farm.

Biocontainment
From the perspective of biocontainment it is important to consider the routes of spreading of the virus from infected premises. Infected birds excrete enormous amounts of virus with secretions from the respiratory tract, conjunctiva and faeces, which ends up
largely into the manure. So likely modes of transmission include moving most of all infected birds and everything that has been in contact with manure. This includes virtually everything that comes out of an infected house or has been at the farmyard. So visitors like veterinarians, consultants and vaccination and service crews or equipment like cages, egg trays, clothes and delivery vehicles may bring viruses along ignorantly. Other potential routes are vermin and insects. Furthermore, potential risks are feed and the truck that delivers it and use of other sources than drinking water supplies (Swayne & Halvorson, 2003). Air-borne transmission has not been identified as a major route of infection (Capua & Marangon, 2000). However, modern poultry houses with large numbers of birds have to use forced ventilation. Dust that is produced within the house particularly when fine wood shavings are used as bedding material can be blown out of the poultry house to distances as far as 1 km. Farms within a close proximity are thus at a higher risk of being infected via this route. Using simple course-dust filters or other dust-removing devices in the air outlet could prevent the spreading of AI via this route and should be pursued.

Obviously, bioexclusion benefits of biocontainment management practices that opt to reduce the risk of carrying AI virus off a premise containing infected birds to a new site (Swayne & Akey, 2005). In most situations these practices focus on preventing movement of AI virus on contaminated equipment, clothing and shoes of farms with infected poultry; preventing movement of infected poultry or their by-products (e.g. manure); or preventing exposure of poultry to wild waterfowl. In many instances, practising biosecurity means controlling the movement of people including restrictions to minimize the number of visitors to farms. Restricting inbound and outbound movements through circumferential fencing of the farm and locking of the gates best achieves this. Other high-risk activities must be managed by using farm-bound equipment or proper cleaning and disinfection of equipment shared between farms, decontamination of clothing and shoes of poultry workers (preferably having farm-bound clothing and shoes with laundering locally), having employees dedicated to one farm, and having strict rules prohibiting employees from owning backyard or recreational poultry or from visiting other poultry farms or establishments.

As for shared employees such as vaccination crews, catchers, feed truck drivers and service personnel, they must diligently practise cleaning and disinfection of equipment (including vehicles), clothing and shoes, and minimize their exposure to the birds. Layer farms should pay special attention to eggs and egg trays. Ideally eggs are collected on farm-bound trays, cleaned and disinfected and then brought to a separate room where they are repacked in transport trays. Transport trays should be cleaned and disinfected at the entrance of the farmyard.

**Discussion**

**Source, infection and spread of AI viruses**

The wild bird population is the sole primary source of all AI viruses. Although in most cases the origin of the virus causing AI outbreaks cannot be established beyond doubt,
all circumstantial evidence points to contacts between wild birds and domestic poultry as the main source. So the question arises why the incidence of AI disease is rising.

The emergence of AI as a veterinary problem in the Western World can be attributed to two main impulses (Lang, 1981). Firstly, since the 1950s the diagnostic methodology for influenza viruses improved tremendously, enabling to actually measure the exposure to AI viruses. Secondly, the drastic change in poultry rearing from the small diversified family farm with a few backyard fowl to the specialized large scale and very competitive agribusiness of today’s poultry business has had a marked influence on the disease situation. The immense concentration and confinement of young susceptible birds created a new and favourable situation for the spread of viral infections. In addition, diseases of relative mildness to the individual bird, which often were overlooked on the family farm, became serious problems on industrial farms when thousands of birds failed to grow or to lay eggs, in accordance with the narrow production performance requirements. The spread of viral infections also profited from the way poultry farming was industrialized after World War II. In many regions the industry grew in an irrational way, consisting of semi-integrated industries like hatcheries, breeders, broiler and layer parent stocks, broiler farms, ready-to-lay pullet and layer farms, feed mills and slaughterhouses, all with separate management. As a result the industry grew without spatial planning, resulting in poultry dense areas and leading to sensitive and dangerous contacts between the different entities of the system.

Temporal and spatial aspects

Outbreaks of HPAI – described by the historical name *fowl plague* – were first described in the Netherlands in poultry in 1924 in the municipalities Achterveld, Scherpenzeel and Woudenberg, the same area that was struck during the 2003 epidemic. The last time HPAI was observed and described in the Netherlands was in 1927 in the same area as in 1924 (Van Heelsbergen, 1927). However, after an absence of HPAI for more than 75 years, a serious suspicion of an HPAI infection on several commercial poultry farms in the Gelderse Vallei, an area in the central-eastern part of the Netherlands with a high density of poultry and poultry farms, was reported on 28 February 2003 (Elbers et al., 2004). The same long interval was observed in the USA. After the last fowl plague outbreak in 1929, it took 46 years before the next HPAI outbreak in Alabama in 1975 (Johnson et al., 1977).

We have to speculate about possible explanations for this phenomenon. First of all there is the fact that AI subtype H5 and H7 infections do occur in wild waterfowl, but at a much lower frequency compared with the non-virulent virus strains and this might be directly translated into a lower probability of occurrence of spillover to domestic poultry. Secondly, on the family farms of those days with only a small number of poultry the disease may have been overlooked. Such a disease became a serious problem on industrial farms when immense concentration and confinement of young susceptible birds created a new and favourable situation for the spread of viral infections.

Experience has learned that it is rather common for HPAI outbreaks to be detected late: at least a week or more after clinical signs have started (Villareal & Flores, 1997; Capua & Marangon, 2000; Selleck et al., 2003; Sims et al., 2003; Elbers et al., 2004).
An important requirement for the development of a large epidemic is an area with a high density of poultry farms. The Italian and Dutch experiences show that an HPAI epidemic in a high-density poultry area is very difficult to control, especially if the detection of the infection is delayed (Capua & Marangon, 2000; Zanella et al., 2001; Stegeman et al., 2004). This points out a considerable risk. In times without AI outbreaks, the farmer and the veterinary practitioner in a disease-free country will be inclined not to report AI-suspect situations. The consequence of not reporting or overlooking AI-suspect cases – because of low specificity of clinical signs – will be a longer period for the virus to spread. The levels of biosecurity management are not tightened by the movement restrictions that are enforced after notification (high-risk period). A longer high-risk period increases the risk of spread of infection to other flocks, especially in high-density poultry areas, which will seriously hamper the eradication of AI after its introduction into a disease-free country.

**Risks for public health**

In the outbreaks of the last 5 years, over 200 millions birds have been affected worldwide, which is a sharp increase compared with the 23 million birds that were affected during the last 40 years before 1999. Major public health concerns were raised for the first time during the outbreak in 1997 in Hong Kong when 6 of the 18 infected persons died of an H5N1 infection after they had been exposed to poultry infected with HPAI H5N1 virus. Since then virus was transmitted to people directly involved in handling poultry infected with HPAI H7N1 virus, with evidence of person-to-person transmission in a few cases. In the Netherlands a veterinarian died of an H7N7 infection during the outbreak in 2003 (Fouchier et al., 2004; Koopmans et al., 2004). During the HPAI epidemic that started in South-East Asia towards the end of 2003, and that was still continuing at the time of processing this paper, a total of 203 cases have been reported in which 113 persons (42 Vietnamese, 14 Thais, 6 Cambodians, 24 Indonesians, 12 Chinese, 4 Turks, 2 Iraqis, 5 Azerbaijans and 4 Egyptians) lost their lives. And this may only be the tip of the iceberg, because only the severe cases have been counted in which people were submitted to the hospital and subsequently diagnosed with AI in the laboratory.

The fear of human infections is twofold. Firstly, infections with HPAI viruses may lead to severe disease, ending in death. Secondly, dual infection with HPAI and human influenza viruses may lead to re-assortment resulting in a virulent virus that effectively transmits from humans to humans. Currently there are no vaccines available against HPAI H5 and H7 viruses. So people can only be treated with antiviral drugs like Tamiflu® (a.i. oseltamivir), either administered prophylactically or therapeutically. Consequently, a re-assorted virus that is effectively transmitted from person to person will spread unlimited and unprecedented throughout the world, causing a pandemic because of the transmission characteristics of human influenza in combination with the intense intercontinental traffic. In case of an influenza pandemic the available production capacity and the amount of antiviral drugs in stock are expected to be insufficient. Moreover, it will take at least 6 months before vaccine production can be started up and the worldwide production capacity is too small to produce
sufficient vaccine in the short time that probably is needed.

Considering the risk of AI for public health it is essential that the poultry industry takes all precautions and uses all resources available to prevent AI outbreaks. This means that any conceivable contact between possibly contaminated animals, contaminated areas around poultry houses and contaminated abiotic vectors on the one hand and domestic poultry on the other must be avoided. Only the highest levels of biosecurity can achieve this. Specifically for free-range poultry, this demand translates, amongst other things, into netting and solid roofing of the free-range area. In case of an outbreak, the disease should be eradicated promptly.

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