Technical Update

LOW PATHOGENIC AVIAN INFLUENZA (LPAI)

INTRODUCTION

Avian influenza (AI) is caused by a type A influenza virus and is distributed worldwide in birds (11). AI viruses are classified by 16 hemagglutinin and nine neuraminidase subtypes. Of these, there are two important classifications: high pathogenicity avian influenza virus (HPAIV) and low pathogenicity avian influenza virus (LPAIV). Low pathogenicity avian influenza viruses circulate naturally in aquatic birds and are the primary reservoir for the disease (11). Spillover of LPAIV from aquatic avian species to commercial poultry or other animals occurs frequently. As LPAIVs replicate and spread in commercial poultry, they can become adapted to the new host, causing disease production losses (11). In this way, LPAIV infections can become endemic in areas of concentrated commercial poultry production, especially operations with poor biosecurity practices or a lack of effective control programs.

When clinically significant, LPAIV infections in laying chickens cause acute respiratory disease and egg production losses. The circulation of LPAIV over time increases the possibility of mutation or reassortment of genes important for virulence, which can result in the emergence of a highly pathogenic avian influenza virus (HPAIV). This occurs mostly commonly with H5 and H7 influenza virus subtypes. HPAIV causes an acute, severe, fulminating disease in most chickens, resulting in high death loss. Economic losses occur directly from disease and indirectly from loss of trade and restrictions. Additional economic hardship comes with the cost of disease control (flock depopulation and clean up) (11).

ETIOLOGY

Avian influenza viruses (AIV) belong to the family *Orthomyxoviridae*, which is responsible for acute respiratory disease in many animal species. All AIVs are classified as *Influenza virus* A, which can be further classified serologically into 16 hemagglutinin (HA) and nine neuraminidase (NA) subtypes. HA and NA are glycoproteins located on the surface of the virus and are important for the virus attachment to host cells during infection (Figure 1). Hemagglutinin is the most important antigen in the bird's immune response against the virus and is used in AI vaccines.

Influenza virus strains are known to have a wide antigenic variation in the genes coding for HA and/or NA glycoproteins. The HA and NA surface glycoproteins are important for the virus to attach and infect the host cells. Influenza viruses are subject to antigenic drift and shift of HA and NA genes. **Antigenic drift** is the result of point mutations in the HA and/or NA genes. Drift antigenic variants can emerge as a result of selection pressure of enzootic LPAIV infections and vaccine immunity. **Antigenic shift** is a more profound genetic change which results from a co-infection of two different influenza viruses in the same cell.

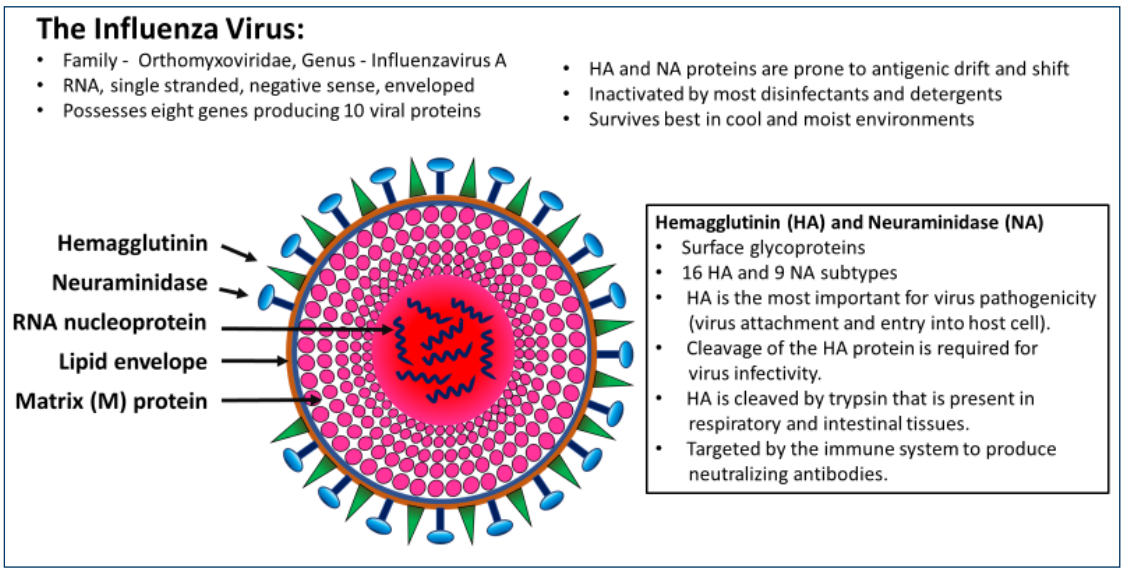

Figure 1. Structure of the influenza virus.

These novel genetic combinations may have increased virulence and transmissibility between avian species, especially from waterfowl to commercial poultry.

AIV of the H5 and H7 subtypes are more prone to antigenic drift and shift, which is why they are of primary interest in global monitoring programs. Sequencing of the H5 and H7 subtypes into LPAIV and HPAIV strains is used by the Office International des Epizooties (OIE) to determine which classification is present in field infection. The OIE is responsible for reporting cases of HPAIV infection to member countries and monitoring ongoing outbreaks. This has implications on the international trade of poultry and poultry products. While not classified as HPAI, it should be noted that there are several examples of non-H5 or H7 viruses that were officially classified by the OIE as LPAIV yet caused significant disease in poultry (8).

Strain	Countries of Occurrence	Clinical Signs / Production Losses	Zoonosis	Comments	Ref.
H9N2 (ongoing)	China, Southeast Asia, Indian Subcontinent, Middle East, North and West Africa	Moderate to severe respiratory disease; egg production drops	Rare mild respiratory symptoms	Endemic in many areas. Prevalent in live bird markets. Continuous circulation with other subtypes increases zoonotic potential	4,9
H6N2, H6N6 (ongoing)	China, Taiwan, Korea, Southeast Asia, South Africa	Moderate to severe respiratory disease; egg production drops	No	Endemic in many areas. Prevalent in live bird markets. Continuous circulation with other subtypes increases zoonotic potential	4,2
H3N1 (2019)	Belgium	Severe respiratory disease, 58% mortality and 100% egg drop	No	Older birds showed more severe clinical signs than young birds	5
H6N1 (2020)	Ireland	Sharp drops in egg production, increased mortality (low); green diarrhea	No	Culling of positive flocks involving >500,000 hens	7

VIRUS SUSCEPTIBILITY TO DISINFECTANTS AND ENVIRONMENTAL CONDITIONS

AIV are inactivated by most of the disinfectants commonly used in poultry facilities due to the presence of a lipid membrane surrounding, referred to as an envelope. Using detergents can break down this lipid envelope, which results in a loss of viral particle infectivity. The virus is inactivated by heat and dryness, but can survive well outside the bird when contained in organic matter (nasal secretions, feces, dust, bird carcasses). The presence of organic matter limits the effectiveness of disinfectants. Cool and moist environmental conditions favor virus survival (11). Composting of bird carcasses and manure for at least 10 days at 60°C (140°F) can inactivate influenza virus (10).

TRANSMISSION

Transmission of LPAIV occurs easily among susceptible birds that encounter nasal secretions, aerosols, or feces from infected birds. Commercial poultry become infected by direct contact with infected waterfowl or materials containing viral particles via a lapse in biosecurity. Secondary transmission between and within commercial poultry facilities typically occurs by mechanical transmission via virus-contaminated materials or movement of infected birds. Important sources of infectious particles are: people, vehicles, equipment, clothing, and footwear. High risk factors for transmission between facilities also include: crews and equipment involved in vaccination, manure handling, and transporting pullets and end-of-lay hens.

Most LPAI infections in aquatic bird species are subclinical (do not produce disease). LPAIV is transported over long distances by infected wild waterfowl during their seasonal fall and spring migrations. During these migrations, waterfowl congregate in high numbers, facilitating wide dissemination of infection.

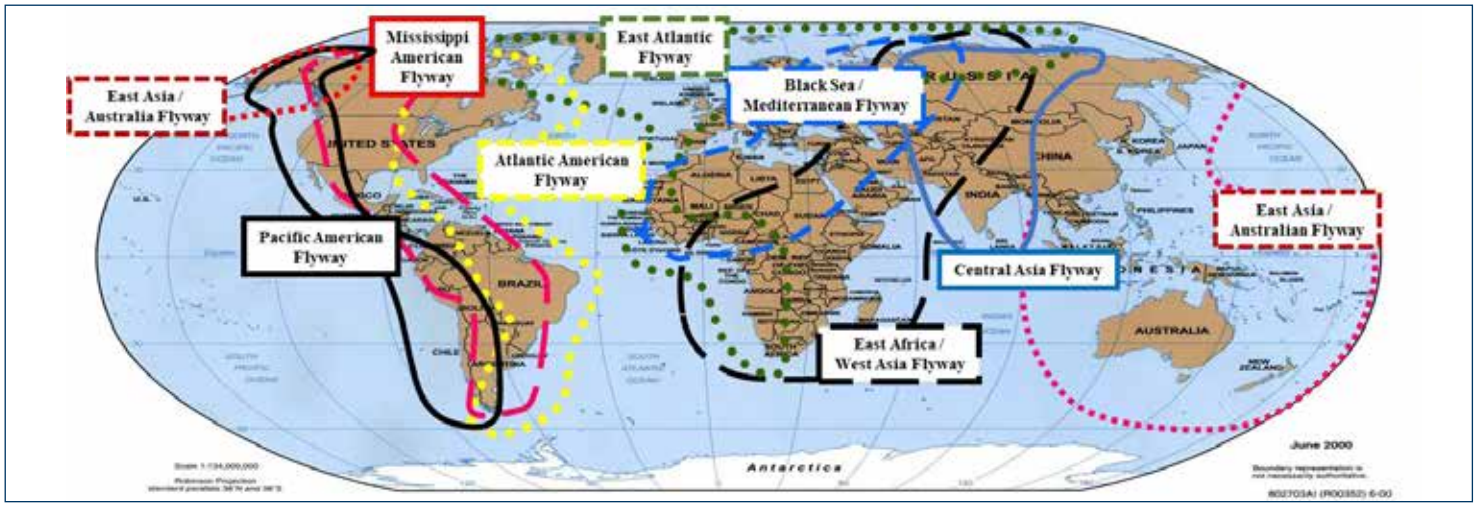

Figure 2. Major waterfowl migratory flyways (1).

Spring migrations in particular bring waterfowl from all the major migratory flyways to nesting areas near the arctic circle (4). This makes it possible for intercontinental spread of influenza viruses where a virus originating from Asian waterfowl can subsequently spread to and infect European and North American waterfowl (6).

CLINICAL SIGNS

The incubation period of LPAIV infection is highly variable and can range between 3 and 14 days in naturally infected birds. This variation in incubation period is dependent on many host, virus, and environmental factors including dose, route of infection, and species involved (11). Many LPAIV infections in chickens do not cause significant clinical signs and are only diagnosed through AI surveillance programs.

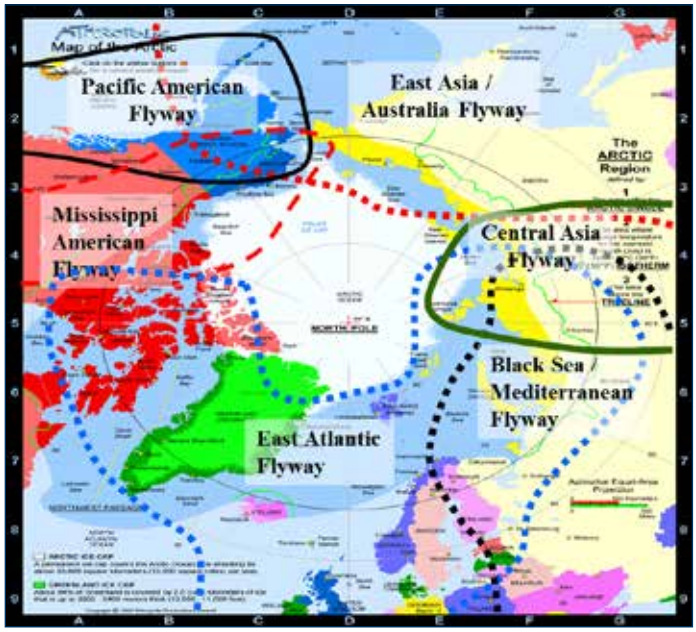

Figure 3. During the spring migrations, the global migratory flyways converge in the Arctic region. For some species of aquatic birds, the nesting areas may overlap (1).

Figure 4. Commercial layer with LPAI exhibiting facial swelling, swollen sinuses and nasal exudate.

Primary clinical signs of LPAIV infection in chickens involve the respiratory and digestive tracts. Clinical signs can vary greatly, but often present as an acute onset of respiratory disease in susceptible populations. Coughing, sneezing, respiratory rales, and facial swelling are frequently observed. Exudate from sinuses may be evident around the eyes and nares, and the infraorbital sinuses are commonly swollen (Figure 4). The digestive tract may be affected, but usually to a lesser extent than the respiratory tract and typically presents as diarrhea. Subcutaneous hemorrhages of the feet and legs may occur as well (Figure 5).

Affected flocks become quiet and appear listless. Naturally, decreased feed and water consumption are the earliest signs of disease, followed by upper respiratory signs and decreased egg production for laying flocks. Egg production and eggshell quality can decrease dramatically, and loss of shell pigmentation can occur in brown or tinted eggs.

Figure 5. Commercial layer with LPAI exhibiting subcutaneous hemorrhage of the legs and feet.

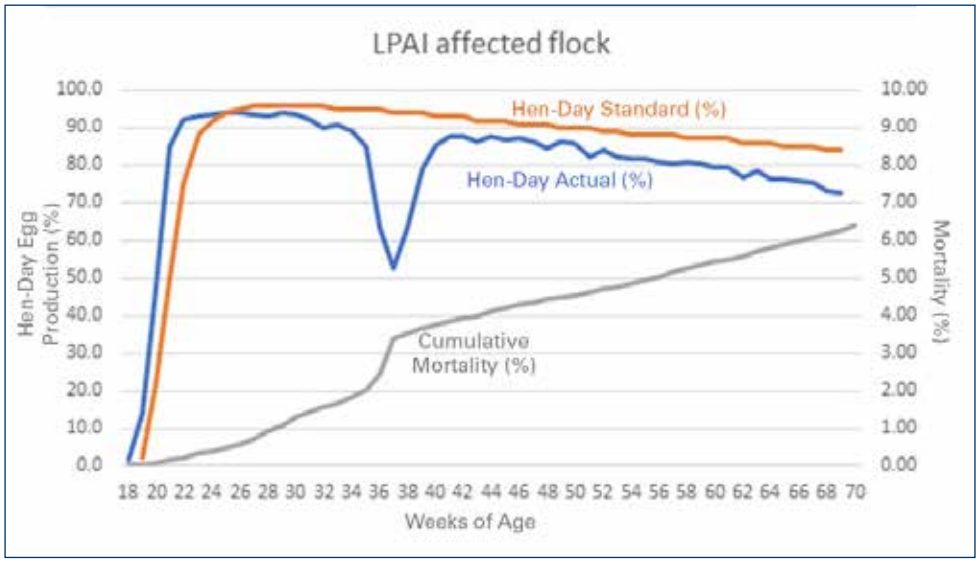

Figure 6. Egg production and mortality in a laying flock infected with LPAIV. The drop in egg production and mortality is highly variable depending on the strain of LPAI, immune status of the flock and the presence of other secondary pathogens.

LPAIV usually causes acute, mild to moderate disease with a pattern of high morbidity and low mortality. Typically, mortality does not exceed 5%, but high mortality has been reported in some LPAI outbreaks (5). Complications from concurrent infections, like *E. coli* or other respiratory pathogens, are common and can result in higher mortality. Mortality is generally higher in young growing birds than in laying chickens; however, this is not always the case.

NECROPSY LESIONS

Most of the pathology occurs in the respiratory, digestive, and reproductive tissues. The mucosal lining of the oropharyngeal area, sinuses, and trachea may appear inflamed and edematous with occasional hemorrhages. A serous to mucoid exudate may be present. Tracheal exudates can form plugs that occlude airways, resulting in suffocation. Pneumonia and airsacculitis may occur, especially when complicating secondary pathogens are present. Petechial hemorrhages surrounding the glands of the proventriculus is a common necropsy finding in layers (Figures 7–9) (11).

Some LPAIV strains are capable of systemic spread to other tissues and as a result, egg yolk peritonitis is a prominent finding with some LPAIV infections (Figure 7). The oviduct might contain inflammatory exudates, with the infundibulum, magnum, and uterus the most affected areas. Later in the disease progression, complete regression of the ovary and oviduct with cessation of egg production is possible. Some LPAIVs spread systemically to the kidneys, resulting in swollen kidneys (nephritis) with accumulation of urates that result in visceral gout. Less frequently, involvement of the acinar cells of the pancreas will result in a "firming" of the gland.

Figure 7. Petechial hemorrhages occurring in the epicardial fat of the heart from a commercial layer infected with LPAIV.

HISTOPATHOLOGY

Acute lymphocytic to heterophilic inflammatory reaction occurs in the affected tissues of the respiratory, digestive, and reproductive tracts. The histopathologic findings are not specific to LPAIV, but constitute supportive evidence when combined with the clinical picture and laboratory findings.

DIFFERENTIAL DIAGNOSIS

LPAIV causes acute respiratory disease and egg production drops like other respiratory pathogens of chickens. Differential diagnoses for LPAIV include infectious bronchitis, Newcastle disease, infectious laryngotracheitis, fowl cholera, and mycoplasmosis. Mixed infections can occur, further complicating diagnosis.

DIAGNOSIS

Detection of viral antigen. The real time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) test is commonly used by laboratories because of its accuracy and short turnover time. Tracheal, oropharyngeal, and cloacal swabs are suitable samples for rRT–PCR testing for a matrix protein common to all type A influenza viruses. Positive samples may be further tested by H5, H7 specific PCR tests.

Detection of viral antibodies. Tests detecting serum antibodies against AIV have been developed and are widely used as screening tests in AI surveillance programs. Antibodies typically appear in infected chickens 5–10 days post-infection. The agar gel immunodiffusion test (AGID), enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition (HI) tests have been developed for determining antibody titers. ELISA is generally more sensitive than AGID or HI tests but with more false positive results.

Isolation of virus. Virus isolation is the definitive test for AIV. Cloacal, oropharyngeal, or tissue swabs from infected birds are inoculated into embryonated eggs at day 9–11 of incubation. After 72 hours, the allantoic fluid is tested for hemagglutinating activity using chicken red blood cells. If hemagglutinating activity is found and determined not to be Newcastle disease virus, which also has hemagglutinating activity, then isolation of an AIV is presumed. Further identification of the HA and NA subtype is performed using subtype-specific antisera. Final identification of an AIV is done at an official government laboratory.

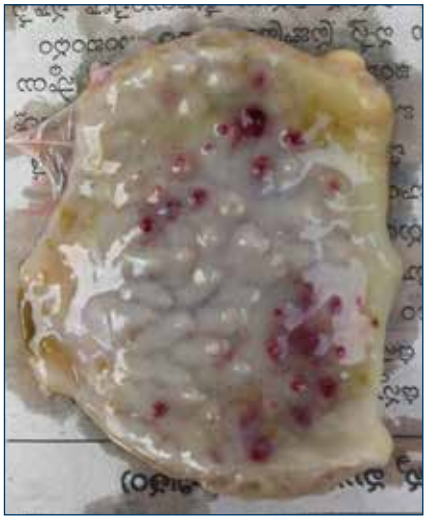

Figure 8. Mucosal petechial hemorrhages surrounding proventricular glands from a commercial layer infected with LPAIV.

Figure 9. Hemorrhages may be found on ovarian follicles and proventricular glands in LPAI affected layers.

Figure 10. Acutely affected hens demonstrating malaise, depression and lethargy.

INTERVENTION STRATEGIES

Effective biosecurity programs prevent contact of poultry with wild aquatic birds, their excretions, and other materials that could contain viral particles. Routine biosecurity measures must be effective enough to prevent an outbreak and contain an outbreak should it occur. Every poultry operation is different and should develop a biosecurity plan that identifies its vulnerabilities for virus introduction and puts into place programs that mitigate these risks.

Movement of birds, people, equipment, feed and materials coming onto a poultry facility must be strictly controlled. Commercial poultry becomes infected by contact with contaminated people, feed or equipment entering the farm. Restrict access to only those people essential to the farm's operation with a change into farm dedicated footwear, clothing and hairnets. Deliveries of feed and materials should be controlled. The vehicles used on farms should be dedicated for use only on the farm. The movement and marketing of old hens must be strictly controlled. On-farm sales of eggs and end-of-lay hens should not occur. The egg trays and bird crates used for product sales to traders should not be returned to the farm or should be fully cleaned and disinfected prior to return to the facility. Use caution and strict control plans when utilizing third-party contractors shared by commercial egg layer companies for vaccinations, moving old hens, pullets, and manure, as these services played a critical role in the spread of AI during the 2014-15 H5N2/H5N8 HPAI outbreak in the Midwestern United States.

Outdoor housing of poultry is an important risk factor and should be avoided during times of wild waterfowl migrations. Free range flocks should be immediately moved to and confined indoors when there are disease outbreaks in the area.

Live bird markets have been involved in several past influenza outbreaks. Live bird markets are often unsanitary and not regulated. It is common that multiple bird species are in close proximity, increasing the possibility of genetic shift and spread of the virus. Limiting the number of bird species sold in a live market, depopulating birds that remain at the end of the day, and cleaning and disinfecting before the next trading day have mitigated some of the risks.

Movement of manure and dead birds pose a significant risk for spreading the virus. Flocks infected with LPAI shed high levels of infectious virus in tissues and manure. When workers and manure handling equipment move between farms, a complete cleaning and disinfection is required. Composting manure and dead birds for 10 days at 60°C (140°F) is an effective way to inactivate influenza virus (10).

Rapid detection of AIV infections. Flocks exhibiting clinical signs consistent with AI infections should be rapidly tested for AI. The diagnostic laboratory should monitor any suspicious cases of respiratory disease. Early detection of AI infected flocks and rapid implementation of intervention strategies to isolate these flocks can prevent further spread. Other poultry farms located near an AI outbreak should be monitored closely.

Eradication of the virus is accomplished by depopulation of infected flocks and isolation of other flocks within an established quarantine area around an outbreak. Flocks are released from quarantine after repeated testing with negative results. This requires strict biosecurity programs, controlled movement of poultry and poultry products to market, and extensive surveillance testing. Eradication of virus has not been achievable in many countries due to the resources required. For many of these countries, the goal is to control AIV infections with vaccination programs and limit the economic impact of the disease.

Table 1: Commercial Vector Influenza Vaccines

Vaccine	Vector Used	Route of Administration	Age of Vaccination	Contraindications
vHVT-AI-H5	HVT (Marek's disease herpesvirus type 3)	Subcutaneous injection	Hatchery	Exposure to another HVT vaccine
vFP-AI-H5	Fowl poxvirus	Subcutaneous injection or wing web inoculation	Hatchery or one day post hatch	Previous exposure to fowl poxvirus (field challenge or vaccine)
vND-AI-H5	Newcastle virus	Spray or eyedrop	One day post hatch	Maternal antibodies to ND

VACCINATION

Avian influenza vaccines have been shown to provide antibody protection against AIV infections. While vaccination does not prevent infection, properly vaccinated birds are protected from the mortality, respiratory disease, and egg production losses associated with AIV infection. Vaccinated birds are more resistant to infection, with less shedding and transmission of infected virus after a field challenge.

The bird's immune system responds to vaccination by producing protective antibodies. The HA subtype of the vaccine is the most important viral antigen in the immune response to vaccination, and so the immunity produced from vaccination is HA subtype-specific. For example, H9 influenza vaccine provides protection against H9 field viruses, but does not protect against the other HA subtypes, such as H3 or H7. For this reason, the AI vaccine selection needs to be antigen-matched to field strains identified from regional disease outbreaks, where the HA subtype of the field strain is known. Subtypes H5 and H7 are commonly utilized because of their increased propensity to become highly pathogenic viruses.

Check local regulations before using AI vaccines. AI vaccination is often under regulation and usually not permitted in countries using a "stamping out" control program.

Inactivated vaccines are the most commonly used AI vaccines. Inactivated vaccines have been developed using H5, H7, H9, and other LPAIV strains taken from field outbreaks. Inactivated vaccines have been effectively used to reduce and, in some cases, eliminate AIV infections in a region. Inactivated vaccines are injected subcutaneously, usually given 2–3 times during the rearing period.

Live recombinant AI vaccines. Live recombinant H5 vaccines have been developed using Herpesvirus of turkeys (rHVT-AIV-H5), fowl poxvirus (vFPV-AIV-H5), or Newcastle disease viruses (vND-AIV-H5) as vectors. Vector vaccines are administered in the hatchery or at one day of age. It is common that vFPV-AI-H5 and vND-AI-H5 are given as a priming vaccination, followed by revaccination with an inactivated AI vaccine. Vectored AI-H5 vaccines provide protection against infection, clinical signs, and mortality caused by H5 field viruses; however, previous exposure to fowl poxvirus interferes with the efficacy of vFPV-AI-H5 vaccines. Similarly, chicks vaccinated with vHVT-AI-H5 vaccine should not receive another vaccine containing HVT. The presence of ND maternal antibodies can interfere with vND-AI-H5 vaccination.

ZOONOSIS

Avian influenza viruses rarely infect people. The most frequently identified subtypes of avian influenza virus that have caused human infections are H5, H7, and H9 viruses.

The most notable is the HPAI H5N1 virus, which emerged from live bird markets in southern China. Workers in these live markets and others near the infected poultry became infected. Transmissibility of H5N1 from infected birds to humans was low, and there was little evidence of human-to-human spread. Human infections of H5N1 virus have been reported in 16 countries in Asia and the Middle East (3).

Transmission from humans to birds is also a rare occurence, seen primarily in turkey flocks (H1N1). Human vaccination for seasonal flu may provide added biosecurity to protect poultry flocks from infection with influenza.

SUMMARY

Avian influenza poses a global threat to egg production facilities, necessitating robust biosecurity programs and diagnostic resources to prevent introduction and possible spread in multi-age complexes or to other sectors of the poultry industry. Most developed countries have created strategic AIV surveillance programs to ensure rapid response in the face of an outbreak, to monitor circulating virus strains, and to ensure trade of product free of avian influenza.

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