Zoonosis Update

Avian influenza and Newcastle disease

David E. Swayne, DVM, PhD, DACVP, DACPV, and Daniel J. King, DVM, PhD, DACVM

Avian influenza (AI) and Newcastle disease (ND) affect various avian species, especially domestic poultry, and are caused by type A orthomyxoviruses and type 1 avian paramyxoviruses, respectively. Clinical manifestations of such infections vary with the virus strain, the host species, and the presence or absence of secondary environmental factors or exacerbating agents. In domestic poultry, infections can be subclinical or induce mild to severe disease syndromes, including respiratory tract disease, decreases in egg production, or a multiorgan systemic disease with a near 100% mortality rate. Severe syndromes are caused by highly virulent specific virus strains termed highly pathogenic AI and velogenic ND viruses. The highly virulent forms of AI and ND are contained on list A of Office International des Epizooties (OIE), the official international organization for animal health and sanitary standards under the World Trade Organization. These highly virulent viruses have severe global impact on poultry health and limit international trade in poultry and poultry products. Furthermore, the viruses that cause highly pathogenic AI and velogenic ND are potential agribioterrorism agents. However, the milder or low virulent forms of AI and ND are more common and endemic in domestic poultry in many parts of the world, but neither are contained on list A or B of OIE. Cases of human infection by AI and ND viruses have been documented, but are rare.

Avian Influenza

Understanding the unique structural and biological features of AI viruses is important in understanding the zoonotic potential for these viruses. Avian influenza virus (family: Orthomyxoviridae, genus: Influenza virus A) is a negative-sense, single stranded RNA virus with a genome composed of 8 gene segments that code for 10 proteins (Fig 1). The 2 surface glycoproteins, hemagglutinin and neuraminidase, are used for epidemiologic study of the virus. There are 15 hemagglutinin (H1 to H15) and 9 neuraminidase (N1 to N9) subtypes; however, the virulence of AI viruses for chickens does not correlate with these subtypes. Most infections with AI viruses (H1 to H15 subtypes) are subclinical or induce mild disease syndromes, whereas a few isolates of H5 and H7 subtypes induce severe, highly fatal disease, termed highly pathogenic AI (historically called fowl plague or fowl pest). The high virulence of H5 and H7 AI viruses in chickens does not correlate with their ability to infect and cause disease in humans.

Diagnosis of AI virus infections in animal and humans has traditionally relied on virus isolation and identification, but this delays obtaining a definitive diagnosis for 1 to 2 weeks. Recently, polymerase chain reaction (PCR) and rapid real-time PCR (RRT-PCR) tests have been developed and used in human and animal field diagnostic situations, respectively. The RRT-PCR was used in the 2002 low pathogenicity H7N2 outbreaks in Virginia poultry and New England live poultry markets. The RRT-PCR test identified AI virus in clinical specimens in < 3 hours and also determined whether the subtype was H5 or H7.

Genetic Change in Influenza Viruses

Influenza viruses have the propensity to change genetically, which contributes to the interspecies transmission and zoonotic potential of AI viruses. Change can occur by 2 mechanisms: random mutations in the
RNA genome, especially in the hemagglutinin, which occur gradually over time; and reassortment of the 8 gene segments that occurs abruptly between 2 influenza viruses that infect a single cell, resulting in progeny that are hybrid viruses. For human Influenza virus A, these phenomena have been termed drift and shift, respectively. The frequency of such occurrences varies with the infection rates in different animal species.6,7

**Primordial Reservoirs and Ecosystems**

Influenza A viruses are endemic, causing infections and disease in humans (*Homo sapiens*), horses (*Equus caballus*), pigs (*Sus scrofa*), and various avian species. Influenza A virus has caused sporadic outbreaks of naturally occurring disease in mink (*Mustela vison*) and various marine mammals (Fig 2).6,11 On the basis of experimental studies12,13 and serologic surveys, rodents (especially mice and hamsters), carnivores, and ungulate ruminants have the potential to be infected with influenza A viruses, but they do not appear to be natural reservoirs. Furthermore, evidence is lacking for them to be biological vectors for transmission of influenza A virus among humans, swine, horses, and domestic poultry—the species that experience endemic influenza.

In birds, the greatest diversity of influenza viruses (all combinations of the 15 hemagglutinin and 9 neuraminidase subtypes) is found in wild birds of the orders Anseriformes (ducks and geese) and Charadriiformes (shorebirds) and occasionally other aquatic bird species (Fig 2). Infections in these species are usually subclinical. These wild bird species serve as the primordial or primitive reservoir of all influenza A viruses and, over long periods, have been the source of influenza viral genes for all influenza A viruses of domestic poultry and mammals.11 Influenza A viruses that infect birds are loosely grouped and referred to as AI viruses, but they are not a separate species or subspecies of Influenza virus A.

Upland game birds, the wild ancestors of domestic poultry of the order Galliformes, are not natural hosts for AI viruses.14,15 However, people have altered the epidemiologic variables of AI viruses by creating new eco-niches via bird captivity and domestication, and demographic variables of AI viruses by creating new ecosystems, pathogenic and nonpathogenic microorganisms can be transmitted within and between avian species and adapt to new host species. Avian influenza viruses have emerged with varying frequencies of infection in birds within 5 human-made systems: 1) captive bird collection and trade systems, 2) live-poultry market systems, 3) backyard and hobby poultry flocks, 4) range-raised commercial poultry systems, and 5) integrated indoor commercial poultry systems. In systems 1 through 3, birds, principally poultry, have been perpetuating host species with endemic infections being common. However, AI infections of commercial poultry (categories 4 and 5) in developed countries are uncommon, but a few epizootics have occurred.

**Transmission and Host Adaptation**

Influenza viruses manifest some host adaptation with frequent and easy transmission between individu-als of the same species or occasionally transmission to closely related species (Fig 2).6 For example, numerous human cases of influenza occur each year, predomin-antly caused by human-origin Influenza virus A strains but infrequently caused by nonhuman-origin Influenza virus A strains, such as swine-origin influenza A viruses.6 On even rarer occasions, AI viruses have been directly transmitted from birds to humans.6 Furthermore, in experimental studies,6 a few AI viruses have shown limited replication in the nasal cavity of humans. The difficulty for transmission and infection of AI virus to humans can be partially attributed to different binding efficiencies of hemagglutinin of influenza viruses for surface cell receptors on avian or human respiratory epithelial cells. The hemagglutinin of AI viruses preferentially binds to N-acetylneuraminic acid-α2,3-galactose linkages on sialoligosaccharide (α2,3 linkage) cell receptors, whereas hemagglutinin of human influenza viruses preferentially binds to N-acetylneuraminic acid-α2,6-galactose linkages on sialoligosaccharide (α2,6 linkage) cell receptors.16 Avian respiratory epithelium has predominantly α2,3 linkage and human respiratory epithelium α2,6 linkage. However, transmission of AI viruses to humans has occurred without this binding preference for α2,6 linkage and may be the result of a particular internal gene combination possessed by specific AI virus strains.16 This shared nonglycoprotein gene constellation was seen in AI viruses that have caused limited human infections in Hong Kong and China since 1997.17

**Avian Influenza Virus Infections in Humans**

Although rare, AI viruses have been transmitted to humans under 2 distinct scenarios: complete AI viruses and individual AI virus gene segments. Infection by complete AI viruses has resulted in true zoonotic infections, but such occurrences have been rarely documented. An even rarer outcome has been coinfection of a person with AI and human-adapted influenza A viruses resulting in a progeny virus that is a hybrid of the different genomes. Such a hybrid virus is not a true zoonotic agent, but a new virus that is adapted for human transmission and infectivity while losing much of its infectivity and transmissibility for avian species.
In toto infection with AI viruses—Human cases of AI virus infection have been documented in the literature on 5 occasions during the past 50 years, but it is probable that many more have gone undetected and undocumented. Three have been isolated cases. In 1959, a 46-year-old male USDA veterinarian developed hepatitis a month after returning from a 2-month trip to various countries in Asia, Africa, and Europe. An H7N7 highly pathogenic AI virus was isolated from his blood by the Centers for Disease Control and Prevention. His recovery was uncomplicated, and no hemagglutination-inhibiting antibodies against the AI virus were detected in acute or convalescent phase sera. In 1978 and 1979, an outbreak of respiratory tract illness and death from low pathogenicity AI virus (H7N7) occurred in harbor seals of the northeastern United States. In association, workers handling the seals developed self-limiting conjunctivitis from the H7N7 AI virus. In England during 1996, a low pathogenicity AI virus (H7N7) was isolated from a 43-year-old woman with conjunctivitis. The women cared for a collection of domesticated ducks that mixed freely with wild ducks on a small lake.

Multiple human cases have occurred twice in Asia. During 1997, infection with a highly pathogenic AI virus (H5N1) in Hong Kong resulted in hospitalization of 18 people with 6 associated deaths. Most of the patients had upper respiratory and gastrointestinal tract symptoms with fever; patients with lethal infections had severe bilateral pneumonia with other secondary complications. Poultry in the live poultry markets were the source of the AI viruses (Fig 3). Depopulation of chickens and other bird species in contact with the chickens on Hong Kong farms and in the live poultry markets was responsible for prevention of additional human cases. The second incident of multiple human cases was between December 1998 and March 1999 when an H9N2 low pathogenicity AI virus was isolated from 5 humans on mainland China and 2 in Hong Kong. Clinical histories were not available for those from mainland China, but fever and respiratory tract disease were reported for the 2 children from Hong Kong. All patients had uncomplicated recoveries. At the writing of this article in early 2003, an additional 2 human cases with 1 associated fatality were reported in Hong Kong in association with H5N1 high pathogenicity AI virus infections. These infections followed a visit by the family to southern China. Furthermore, the family experienced 3 additional cases of respiratory tract disease with 1 associated death, but the H5N1 virus was not known to be the cause of these illnesses at the time of publication. Beginning in February 2003, an outbreak of highly pathogenic H7N7 AI has been ongoing in egg-laying chickens in the Netherlands. On Apr 17, 2003, the first human fatality from the H7N7 AI virus was reported. The case was in a 57 year-old healthy veterinarian who on Apr 2, 2003, had visited a poultry farm that had H7N7 AI virus infected chickens. The man developed a high fever and severe interstitial pneumonia. The H7N7 AI virus was isolated from bronchial lavage fluid and lung tissue. As of Apr 30, 2003, 82 cases of H7N7 AI virus infections in people have been reported. The majority of patients experienced self-limiting conjunctivitis without pulmonary involvement. Most of these human cases resulted from contact with H7N7-infected live chickens, but in 3 cases, there was evidence of transmission from poultry workers to family members. Most of the recent human infections have resulted from non-compliance with personal biosafety measures to reduce human exposure to H7N7 AI virus; e.g. wearing of gloves, gowns and masks. Fortunately, no co-infections with the H7N7 AI virus and a human influenza A virus have been detected. This outbreak emphasizes the need for continuing cooperation between public health and veterinary medical communities in controlling AI when it has a zoonotic potential.

Reassortment of avian- and human-adapted Influenza virus A—Avian influenza viral genes have rarely appeared in human-adapted influenza A viruses; however, they have resulted from reassortment of gene segments between more than 1 influenza A virus. Such a reassortment event is rare, but consistent with the long spans between the emergence of new human pandemic influenza viruses. Two reassortment events between AI and human-adapted influenza viruses caused pandemics of human influenza during 1957 and 1968. Nucleotide sequence data indicate the 1957 (H2N2) human pandemic influenza viruses resulted from reassortment of 3 (hemagglutinin, neuraminidase, and polymerase B1) AI viral genes with 5 (matrix, polymerase B2, polymerase A, nonstructural, and nucleoprotein) human influenza viral genes. The 1968 (H3N2) pandemic virus resulted from reassortment of 2 (hemagglutinin and polymerase B1) AI viral genes with 6 human influenza viral genes. Direct infection of humans with AI viruses makes such a reassortment event feasible. At the end of 1997, depopulation of infected poultry in Hong Kong before the onset of human influenza season may have forestalled a reassortment event and prevented another human influenza pandemic. Swine have been proposed as a “mixing vessel” for coinfection by influenza viruses from birds and mammals, with reassortment of gene segments and development of hybrid strains having the ability to infect people and other mammals.
Risk of AI Viruses for Humans

The general risk of AI viruses infecting humans has been extremely low, and AI virus strains vary in ability for transmission to and infection of humans. For example, during the 1983 to 1984 United States H5N2, 1997 Italian H5N2, and 1999 to 2000 Italian H7N1 AI outbreaks, individuals with high occupational exposure to infected poultry, such as veterinarians and depopulation crews, lacked either virologic evidence for replication of the AI viruses in the upper respiratory tract or serum antibodies against the respective AI viruses. 25,40,41

By contrast, the 1997 Hong Kong H3N1 AI viruses infected and caused severe illness and hospitalization of 18 people with 6 resulting deaths. Although the fatality rate was high in the limited number of people hospitalized, serologic studies40,41 indicated infection was more widespread in people with high occupational risk, such as poultry workers (17% seropositive rate), but such infections were not accompanied by specific clinical illness or death. Intense poultry exposure, especially slaughtering operations or chickens with clinical disease, was associated with development of antibodies. 40

Minimal evidence exists of human-to-human transmission of the Hong Kong H5N1 AI viruses.25,42,43 Overwhelming evidence supports that most of human infections resulted from direct poultry-to-human transmission.25 For the hospitalized patients, a risk factor was exposure to live poultry at a retail poultry stall or a market selling live poultry the week before.25 Most likely, the AI viruses were obtained from contact with poultry respiratory tract or fecal secretions that contained high concentrations of the AI virus. Handling, cooking, or consuming poultry meat were not risk factors for human infections with Hong Kong H5N1 AI viruses.25

Experimental studies44,45 using mouse and hamster models have emphasized the unique potential of a few AI viruses for infecting mammals. Three 1997 Hong Kong H5N1 AI viruses caused infection, severe disease, and a 100% mortality rate in mice, whereas A/chicken/Scotland/59 (H5N2), A/turkey/England/91 (H5N1), A/chicken/Queretaro/7653-20/95 (H5N2), A/chicken/Italy/87 (H5N2), and A/environment/Hong Kong/437/99 (H5N1) AI viruses were nonpathogenic or mildly pathogenic for mice.46 All 8 AI viruses were highly pathogenic for chickens. In another study45 using hamsters, two 1998 Hong Kong/China H9N2 AI viruses that were mildly pathogenic for chickens induced highly lethal infections in hamsters without the need for laboratory adaptation to the hamster model. Extrapolation of results from such animal studies emphasizes the potential for some AI viruses to infect and cause severe disease in humans. Furthermore, experiments with human volunteers found that most mildly pathogenic H1N1 and H3N2 AI viruses did not replicate in the upper respiratory tract.17 A few H4N8, H10N7, and H6N1 AI viruses had limited replication with development of mild upper respiratory tract signs, but without detectable primary immune responses.25

Recent Avian Influenza Events in the United States

During 2002, an outbreak of low pathogenicity H7N2 AI occurred in chickens and turkeys in Virginia on 197 commercial farms and on a more limited basis in North Carolina and West Virginia.46 The AI viruses isolated were closely related to low pathogenicity H7N2 AI viruses that have circulated in the live poultry markets of New England since 1994. The H7N2 AI virus was eliminated from poultry in the commercial sector and live poultry markets because of its negative impact on US poultry exports and because of accumulative genetic changes in the hemagglutinin gene that positioned the virus to become highly pathogenic for poultry with only 1 additional nucleotide substitution. No cases of conjunctivitis or other influenza-like illnesses were attributed to the H7N2 AI virus in people with high exposure risk (ie, working on the eradication task force).

NEWCASTLE DISEASE

The first reported human infection with ND virus (NDV) resulted from a laboratory accident that occurred during 1942.47 In 1926, the first outbreaks of ND occurred in chickens in Indonesia and England and after the recognition of NDV as the etiologic agent of ND. The disease name was chosen to differentiate it from fowl plague in chickens, which is now known as highly pathogenic AI.48 Other synonyms to identify ND have included avian pneumoencephalitis and pseudofowl pest.

Etiology

Isolates of NDV are classified in the family Paramyxoviridae, genus Avulavirus, and comprise the avian paramyxovirus type 1 (APMV-1) serotype. Although all isolates are of 1 serotype, they do vary in virulence from those that cause high mortality rates in susceptible birds to those that induce only subclinical infections. The clinical signs vary with the virulence of the strain, the avian species infected, and the predilection of the infecting virus for the respiratory and gastrointestinal tracts or CNS. Because of the large difference in virulence among NDV isolates, a simple classification of strains was based on the observed clinical signs and lesions in chickens. High death rate with a high frequency of hemorrhagic intestinal lesions was evident in the earliest outbreaks of high virulence NDV infections. Subsequently, outbreaks were reported in chickens that developed abnormal CNS signs but no hemorrhagic lesions. Mortality rates were low to high, and the isolated viruses had moderate to high virulence. Finally, infections with low virulence viruses induced mild respiratory tract disease or no illness at all. The current widely used live virus ND vaccines were derived primarily from these low virulence isolates; however, in countries where virulent strains are still endemic, viruses of moderate virulence are used in vaccination programs to control ND.49 New procedures for differentiation of NDV isolates have largely replaced classification by disease form other than for descriptive clinical reports. Molecular epidemiologic studies are possible because monoclonal antibodies detect antigenic differences,49 and nucleotide sequence analysis detects phylogenetic differences49 among strains within the APMV-1 serotype.

Recent advances in molecular characterization of NDV have provided the basis to determine the poten-
tial virulence of NDV isolates by nucleotide sequence analysis of the NDV fusion protein gene to deduce the amino acid sequence of the protein. The fusion protein of NDV functions to fuse virus and cell membranes, thus facilitating entry of the virus nucleocapsid into the cell and initiation of infection. The critical region for this activity is the fusion protein cleavage activation site composed of a sequence of amino acids that must be cleaved by proteases to render the virus infectious. The number of basic amino acids at the cleavage site determines the type of cellular protease effective in cleavage of the fusion protein; therefore, determining the amino acid sequence at that site is the basis for predicting the potential virulence of an NDV isolate for poultry.

The fusion protein cleavage site of low virulence viruses contains fewer basic amino acids and is cleaved by a protease in a restricted number of cells. Thus the lentogenic strains, like the vaccines B1, La Sota, and VG-GA, typically multiply most efficiently in the mucosa of the respiratory tract or, in some cases, the intestinal tract. The more virulent viruses, the mesogens and velogenes, contain more basic amino acids at the cleavage site, and their fusion protein is cleaved by proteases in a wide range of cells and tissues in addition to the respiratory and intestinal tracts. The molecular differences in the fusion protein that differentiate viruses of low virulence from the more virulent viruses generally parallel the results of inoculation of susceptible chickens, the standard method of determining the virulence of NDV isolates for chickens.

Clinical Disease in Humans

Humans are among the many species that can be infected with NDV in addition to at least 241 avian species. The most common sign of infection of humans is conjunctivitis that develops within 24 hours of NDV exposure to the eye. In the first reported case, it was also noted that the preauricular lymph node on the involved side was swollen and tender. General symptoms of headache, discomfort, and slight chills occurred within 48 hours of the initial infection, but lasted for only 24 hours. Local treatment was used to relieve symptoms of the conjunctivitis, which cleared completely within a week. Virus was reisolated from the lacrimal fluids of the infected individual, who became seropositive within 2 weeks after the exposure. Subsequently, reported infections were similar and usually resulted in self-limiting eye infections characterized by unilateral to occasionally bilateral conjunctivitis, edema of the eyelids, congestion of the conjunctiva, tearing, and pain without involvement of the cornea. Rarely, NDV infection has resulted in symptoms of fever, chills, headache, pharyngitis, depressed appetite, photophobia, and general apathy. A history of aerosol exposure was associated with most of the individuals who developed a generalized infection.

Many of the documented cases involved infections in laboratory workers who accidentally splashed high-titer NDV-infected egg fluids into their eyes, veterinary laboratory diagnosticians who performed postmortem examinations on infected birds or handled infectious tissues, workers in poultry processing plants, and poultry vaccination crews. The absence of many recent reports suggests that such infections may be commonplace and that when they occur, they are mild and self-limiting. The use of personal protective equipment and biological safety cabinets in laboratories has undoubtedly reduced the exposure of current laboratory workers.

The virulence of NDV isolates does not appear to differ for humans in contrast to the wide difference in virulence for chickens. Experimentation with a virulent field isolate was the cause of the initial human infection, but similar signs can result from infections with low virulence NDV strains, such as B1, which are commonly used as ND vaccines. No evidence exists to support human-to-human transmission, but the potential for human-to-bird transmission exists. Because of the mild, self-limited infection of humans by NDV, the risk of substantial illness and pandemics is unlikely. Multiple treatments of humans with NDV administered by inhalation or IV without substantial adverse effects are further evidence that the risk of severe illness is low, although low-grade fevers that subsided in 24 hours did occur after inhalation therapy in 8 of 23 vaccine-treated patients. In the inhalation studies, it was also noted that mild conjunctivitis was a consequence of accidental eye inoculation.

Newcastle Disease Virus as Cancer Therapy for Humans

Alternative therapies have been proposed and used in people in whom conventional cancer therapy has failed to stabilize or regress existing tumors. The observation that tumor regressions occurred during natural virus infections or immunizations was the basis of preliminary studies that have led to the use of at least 10 viral species in current clinical trials. A case history involving a poultry farmer whose metastatic gastric carcinoma underwent regression coinciding with an outbreak of ND in his chickens led to the application of an attenuated NDV vaccine for treatment of a few terminal cancer patients with favorable results. Various NDV strains have been used in cancer treatment, because antitumor responses have differed among the isolates used. The strains Ulster and Italien are readily identifiable poultry isolates of low and high virulence, respectively, whereas NDV 73-T was selected from multiple passages in Ehrlich ascites tumor cells. Intracerebral inoculation of day-old chicks with 73-T induced paralysis and death, an indication that it is an isolate of moderate to high virulence.

The beneficial effects of the treatment of some cancer patients with NDV may be attributed to several mechanisms. First, NDV has potential antitumor activity via direct growth inhibition and oncolytic action on various cancer cells. Experimentally, NDV replicated and killed various human and rat neuroblastoma cells, but did not replicate and had no cytotoxic effect on normal human fibroblasts. Regression of a glioblastoma in a boy has been reported after daily IV administration of live NDV MTH-68. In a placebo-controlled study of 59 patients with advanced cancers, twice weekly inhalation therapy with live MTH-68 induced regression and no progression or malignant tumor stabilization in 18 patients (35%) of the NDV-treated group, compared
with only 2 patients (8%) with similar favorable results in the placebo-treated controls. The mechanism for NDV oncolytic activity is unknown, but NDV has been shown to be a potent inducer of α tumor necrosis factor (TNF-α) production by human peripheral blood mononuclear cells and enhance sensitivity of the tumor cells to the cytolytic effect of TNF-α.19

In addition, NDV may provide anticancer therapy through pleiotrophic modification of the patient’s own immune response against the tumor rather than direct oncolytic activity. A human tumor vaccine prepared from patient-derived autologous live tumor cells, which were inactivated with γ-irradiation and then infected with the nontoxic strain NDV Ulster, has been shown to have advantages over autologous NDV oncolyses as a postoperative immune adjuvant.62

It is the opinion of the authors that NDV isolates of moderate to high virulence prepared for cancer therapy present a greater risk for poultry than for humans should inadvertent exposure occur.

Recent Outbreak of ND in US Poultry

An outbreak of exotic ND that began in privately owned backyard flocks in southern California in October 2002 spread to commercial poultry operations in that region by late December 2002. The cause of conjunctivitis in 2 workers involved in the effort to eradicate the exotic ND outbreak is being investigated.39 The low prevalence of suspected human NDV infections among the > 600 workers involved in the identification, euthanasia, and disposal of infected flocks is similar to earlier reports32 of NDV infections in humans. Because NDV infections of humans have a history of being mild and self-limiting,1 the concern is much greater that infected individuals may transmit the virus through their contact with susceptible birds and poultry and thus extend the outbreak than is the concern that such infections will become a human health problem. It appears that human infections with highly pathogenic Al virus, as have recently occurred during an outbreak of disease in poultry in Holland, are a greater concern at the present time.59

References
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