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Orthomyxoviridae

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This is a family of negative-sense, single-stranded RNA viruses. They are smaller than the paramyxoviruses and their genome is segmented (7 to 8 segments) rather than consisting of a single piece of RNA. Influenza viruses are the only members of Orthomyxoviridae.
Viruses of this family have a predilection for the respiratory tract, but usually do not cause a serious disease in uncomplicated cases. Exceptions are human infections with viruses of avian origin. Principal viruses of veterinary importance are type A influenza viruses, which cause equine, swine, and avian influenza.

Viral Characteristics

- Viruses have a segmented single-stranded RNA genome, helical nucleocapsids (each RNA segment + proteins form a nucleocapsid) and an outer lipoprotein envelope. See Fig. 20.1.
- The segmented genome facilitates genetic reassortment, which accounts for antigenic shifts. Point mutations in the RNA genome accounts for antigenic drifts that are often associated with epidemics. In either case, the changes are frequently associated with the HA (hemmaglutinin) and NA (neuraminidase) antigens.
- The envelope is covered with two different kinds of spikes, a hemagglutinin (HA antigen) and a neuraminidase (NA antigen). In contrast, the hemagglutinin and neuraminidase activities of paramyxoviruses are in the same protein spike.
- In the laboratory, the virus replicates best in the epithelial cells lining the allantoic cavity of chicken embryos.
- The viruses agglutinate red blood cells of a variety of species.
- Replication takes place in the nucleus.
- The viral RNA-dependent RNA polymerase transcribes the negative-sense genome into mRNA.
- Influenza viruses are labile and do not survive long on premises.

Figure 20-1. Orthomyxoviridae (80 to 120 nm). Helical nucleocapsid surrounded by an envelope with hyluronidase and nuraminidase spikes. - To view this image in full size go to the IVIS website at www.ivis.org . -

Immune Response
The host immune response to influenza viruses includes:
- *non-specific immune response*: the release of interferons by infected cells aids in preventing viral spread to neighboring cells.
- *humoral immune response*: IgA in the upper respiratory tract and IgG in the lower respiratory tract. These antibodies are typically directed against the HA and NA antigens.
- *cell-mediated immune response*: cytotoxic T lymphocytes are important in recovery.

Classification
The family consists of four genera:

Influenza virus A: Viruses cause avian, equine and swine influenza; associated with both epidemics and pandemics; both antigen shift and antigen drift noted. High antigenic variability in the surface glycoproteins HA and NA.
Influenza virus B: Members infect only humans; associated with epidemics; antigenic drift noted.
Influenza virus C: Viruses cause mild, sporadic respiratory infections in humans. May also infect swine.
"**Thogoto-like viruses**": The two species Thogoto and Dhori viruses are tick-borne viruses recovered from cattle, camels and humans in regions of Asia, Africa and Europe. They are not considered to be of pathogenic significance for animals.

Antigenic Composition
Knowledge of the antigenic nature of influenza viruses is necessary for an understanding of the epidemiology of influenza.
- The internal proteins consist mainly of nucleocapsid protein (NC), some matrix proteins (M1) and three polymerases (PA, PB1 and PB2). The proteins NC and M1 determine type specificity. Even being internal, these proteins (or peptides derived from them) may elicit cytotoxic T cells that are important in recovery from infection.
- The nucleoprotein antigen (A, B, C) determines the virus type. The HA and NA antigens determine subtypes.
- The hemagglutinin (HA) is an envelope antigen (spike) that can attach to erythrocytes and cause agglutination. It is responsible for the attachment of the virion to cell surface receptors (neuraminic acid, sialic acid). If blocked by antibody, attachment of the virus to a susceptible cell is prevented; thus it is very important in protective immunity mediated by neutralizing antibody. A hemagglutination-inhibition titer of 1/40 is considered to be protective.
- Neuraminidase is an envelope protein whose enzymatic activity results in the liquefaction of mucus thus contributing to viral spread. Specific antibody slows down the spread of virus. Neuraminidase also cleaves neuraminic acid to release progeny virus from the infected cell.
- Influenza viruses are designated as follows: type/place/time of isolation/H and N content. In birds, there are approximately 15 H antigens (H1 - H15) and 9 N antigens (N1 - N9), which can be found in all possible combinations. An example would be H7 N3. Therefore, the type A virus: A/Bangkok/3/79 (H3N2) denotes, respectively, type A, isolated in Bangkok, local laboratory designate of number 3, first isolated in 1979, and envelope antigens of H3N2.

Antigenic Variation
In brief there are two kinds of antigenic changes:
Antigenic shifts: These are major changes based on reassortment of segments of the genome. In reassortment, entire segments of RNA are exchanged between two viruses infecting the same host, each of which codes for a single protein, e.g., the hemagglutinin. As a result of co-infection by two viruses, a third one may arise.
Antigenic drifts: These are minor changes caused by point mutations in the genes encoding the HA and NA glycoproteins.

Genetic Basis for Antigenic Variation
- The genes of the type A viral hemagglutinin and neuraminidase are polymorphic, subject to extensive variation. This not the case for types B or C.
- The HA and NA genes of types A and B viruses undergo point mutations. When developing a vaccine, the effect of change can be determined by the reciprocal inhibition test. As a result of the change, the immune response generated against the vaccine HA or NA is now less effective against the mutated (variant progeny) HA or NA.

Influenza A
Equine Influenza

Cause
Equine Influenza virus A. The immunologically distinct subtypes involved are usually A/equine/Prague/1/56 (H7N7) or A/equine/Miami/2/63 (H3N8). These are also referred to as influenza A/equine1 and influenza A/equine 2. They are also referred to as Type l or A Equi-1 (Prague) and Type 2 or A Equi-2 (Miami). New variants resulting from antigenic drift appear to be infrequent. All recent and current outbreaks have been attributed to A Equi-2.

Occurrence
A frequently occurring highly contagious disease of horses, mules and donkeys throughout the world excepting Australia,

New Zealand and Iceland. The last recorded outbreak of A Equi-1 was in 1980. Several variants of A Equi -2 attributed to antigenic drift have been reported.

Transmission
By direct and indirect contact. Droplet infection is the primary means of spread.

Clinical & Pathologic Features
Infection is by the respiratory tract and the virus replicates and destroys epithelial cells of the upper respiratory tract. Clinical signs begin in about 1 - 3 days post-infection. The most common signs are fever, depression, anorexia, and coughing. Most infected horses exhibit some degree of ocular discharge and photophobia, and some may develop edema of the legs.
Pneumonia occasionally occurs if the infection is complicated with secondary bacterial infection. In the absence of stress and bacterial complications, recovery is uneventful in about 7 - 10 days, although the ability to work may be impaired for several weeks.
The severity of the disease depends considerably on immune status and age. Young horses are particularly susceptible.

Diagnosis
- Clinical specimens: Nasal and ocular swabs during the acute phase, acute and convalescent sera.
- Equine influenza is often diagnosed clinically based on its sudden onset, rapid spread, high fever, and coughing.
- Laboratory confirmation is obtained by isolation of the virus in embryonated chicken eggs or by the demonstration of a significant increase in specific antibody between acute and convalescent sera using hemagglutination inhibition (HI) tests.

Prevention
- Killed vaccines with or without adjuvants are used to prevent the disease. The protection is short and periodic boosters are required.
- Clinically ill animals should be isolated to prevent spread. Cleaning and disinfection should be applied to reduce spread.
- A new intranasal, modified-live virus vaccine claims to protect for six months.
  Antigenic variability should be considered in the preparation of vaccines.

Avian Influenza
(Fowl plague)

Cause
Influenza A virus avian. There are 15 antigenic groups based on hemagglutination inhibition and nine based on neuraminidase. The many strains of virus infecting waterfowl provide sources for new mammalian strains, e.g., strain H5N1 a highly pathogenic avian virus caused influenza with high mortality in humans in Hong Kong. Subtypes H5 and H7 have caused serious outbreaks of avian influenza in commercial flocks of chickens and turkeys. When these pathogenic strains are identified, premises are quarantined and infected flocks slaughtered.

Occurrence
The disease occurs widely in chickens, ducks, turkeys, quail, pheasants, other fowl and particularly in waterfowl. Avian influenza has recently swept southeast Asia (~10 countries). Since 2003, tens of millions of chickens have been destroyed to prevent spread of the virus. In January 2005 alone 1.2 million chickens were destroyed. More than 20 people died. Importation of birds, including pet birds, from certain countries was banned. Avian influenza occurs periodically in several East coast states of the United States. Outbreaks have been attributed to migratory waterfowl.

Transmission
Avian influenza is highly contagious and spreads rapidly. Transmission is by droplet infection, direct and indirect contact (fomites). Migratory waterfowl with subclinical, enteric infections can excrete virus for long periods. They are a frequent source of infection for domestic poultry.

Clinical & Pathologic Features
There are numerous strains or subtypes of avian influenza viruses. Most of these viruses are associated with subclinical or mild to moderate respiratory infections characterized by coughing and sneezing. Sinusitis may develop and infected birds often experience decreased egg production. Concurrent bacterial infections exacerbate the disease.
The highly pathogenic forms of avian influenza (fowl plague) cause severe generalized disease with high mortality in commercial flocks. Deaths may occur as early as 24 - 48 hours after the onset of clinical signs. The latter are similar in many respects to velogenic viserotrophic Newcastle disease, including occasional neural disturbances. Comb and wattles are cyanotic and there may be edema of the head region with coughing, gasping, blood-stained oral and nasal discharges, and diarrhea.

Lesions include hemorrhages and congestion of serous and mucous membranes, consolidation of lungs, and caseation involving the air sacs. Focal necrosis may be noted in the skin and internal organs.

Diagnosis
- Clinical specimens: Whole birds killed *in extremis* or lung, trachea, air sac, kidney, spleen, and serum.
- The virus is isolated by inoculation of embryonated eggs and identified using virus neutralization and hemagglutination inhibition tests with specific antisera. Differentiation from Newcastle disease is carried out with specific immune sera. Allantoic fluid from infected chicken embryos agglutinates RBCs.
- Sera may be tested for antibody using an agar gel diffusion test and embryonated egg antigen. A similar procedure tests for antigen in embryonated egg (chorioallantoic membrane) using known positive influenza A serum.
- In the USA, isolates of avian influenza virus may be sent to the National Veterinary Services Laboratory for serotyping and pathogenicity studies.

Treatment
Amantadine hydrochloride has been used to reduce the severity of influenza in some avian species, but amantadine-resistant viruses have been noted.

Prevention
- Avian influenza is a reportable disease in some countries including the USA. Confirmed outbreaks of highly virulent avian influenza (fowl plague) are dealt with by strict quarantine and slaughter.
- Special efforts are made to prevent exposure to migratory waterfowl, e.g., along the Atlantic coast of the USA.
- In those countries where avian influenza viruses are endemic, or at risk of introducing the virus, and maintaining closed flocks is difficult, inactivated vaccines are used. The continuous emergence of new subtypes complicates immunization.
- Appropriate antibiotic therapy may be beneficial in controlling secondary bacterial infections in mild forms of avian influenza.

Public Health Significance
As stated earlier the pathogenic avian strain H5N1 causes severe influenza in humans. There have been a number of reports of pathogenic avian strains causing serious and even fatal influenza (~72% mortality rate) in humans in southeast Asia where there is often close contact between large numbers of poultry and people. There is real concern that H5N1 strains from poultry could cause catastrophic epidemics or even a global pandemic of human influenza in the near future. To cause human epidemics, the virus would have to develop the capacity for person-to-person transmission. The human influenza viruses causing the pandemics of 1918, 1957 and 1968 contained gene segments closely related to those of avian influenza viruses.

It is of interest that in October 2005 scientists at the Centers of Disease Control and U.S. Armed Forces Institute of Pathology reconstructed the 1918 flu virus. The gene sequence of the reconstructed virus is based the gene sequence of the original virus isolated from a flu victim buried in the Alaskan permafrost. Based on gene analysis, three of the eight virus genes appear to be associated with human host adaptation of the avian virus. Interestingly, the reconstructed 1918 virus kills the cells of fertilized eggs used for propagation of flu viruses, similar to the more recent Asian bird flu viruses. Typical human influenza viruses do not kill these cells.

Swine Influenza
(Swine flu, Hog flu)

Cause
Influenza virus A. Subtypes H1N1 and H3N2 have been frequent causes of swine influenza. More virulent variants of H1N1 have appeared in recent years. The H1N2 subtype has also been implicated as a cause of acute swine influenza. It has been suggested that all porcine influenza viruses were derived originally from birds.
Secondary infection with *Haemophilus parasuis* and other bacteria may contribute to a more severe disease.

Occurrence
Swine influenza occurs worldwide. Influenza strains from swine may produce serious infections in humans, other mammals, and birds.

Transmission
Swine influenza is widespread, occurring most commonly in the colder months. Aerosol droplets, contact, and fomites are the means of spread. In swineherds where the virus is endemic, young susceptible pigs are continually infected, thereby maintaining the virus. Explosive outbreaks of acute disease occur when the virus is introduced into susceptible, naive herds.

Clinical & Pathologic Features

Morbidity is high but the mortality is usually no greater than 2%. Virus infection is mild without secondary bacteria. In a typical outbreak, there is an incubation period of about three days followed by an acute onset of respiratory distress with rapid respiration, coughing, anorexia, and prostration. The clinical course is usually 2 - 6 days with rapid recovery in uncomplicated cases. With secondary invaders and particularly *Haemophilus suis* the disease is much more serious and some deaths may occur.

Necropsy in acute cases discloses edematous mediastinal lymph nodes and pneumonic lesions usually limited to the apical and cardiac lobes. Affected areas are firm and purplish in color and there is often a sharp line of demarcation between normal and affected tissue. Exudative bronchitis and interstitial pneumonia are common microscopic findings.

Diagnosis

- Clinical specimens: Nasal swabs and lungs, acute and convalescent sera.
- Given the often mild character of the disease, laboratory diagnosis may not be sought.
- A presumptive diagnosis is made on the basis of clinical and pathologic findings. Confirmation requires isolation and identification of the virus, demonstration of seroconversion or detection of viral infected cells in frozen sections of lung tissue by immunofluorescence.
- Swine influenza virus is most easily isolated by the inoculation of embryonated eggs via the allantoic cavity.

Prevention

- Swine influenza virus is usually introduced into herds via replacement stock or returning show animals.
- Vaccination is not practiced.
- In the event the disease is particularly severe, antibiotics may be used to control secondary bacteria.
- Recovery from swine influenza infection confers immunity, but the duration is probably less than a year.

Canine Influenza

(Dog flu)

Cause

Influenza A virus, closely related to subtype H3N8 and presumed to have been acquired from a horse(s). Subtype H3N8 virus is a frequent cause of equine influenza.

Occurrence

Dog flu first occurred in greyhounds in Florida, USA in January 2004. Of the dogs affected by the outbreak about 30% died. The disease was first seen in race tracks but has since spread to veterinary clinics, boarding facilities and animal shelters. Infections have also been confirmed in New York and a number of other states.

Older dogs and puppies may be more severely affected.

Transmission

This is mainly by infectious aerosols resulting from coughing but also indirectly by fomites.

Clinical Features

It appears that the disease is less severe than earlier thought and about 80% of those infected come down with a mild disease. The incubation period is 2 - 5 days. Among the clinical signs are fever, coughing, dyspnea, anorexia, depression and mucoid to purulent nasal discharge. In mild cases recovery is within 1 - 3 weeks. Pneumonia with secondary infection is the most common and serious complication. The fatality rate has ranged from 5 - 8% in the early outbreaks among greyhounds but somewhat less in later outbreaks.

Diagnosis

Dog flu can only be distinguished from other respiratory infections, such as kennel cough, by laboratory means.

- Specimens: Nasopharyngeal swabs taken within 72 hours of the appearance of signs; paired serum samples with a three-week interval.
- Virus isolation is readily accomplished in chicken embryos.
- Viral antibodies are identified by hemagglutination-inhibition or virus neutralization.
- There are several rapid commercial tests for antigen used for the diagnosis of human influenza which may have application for the canine disease. A rapid human influenza A kit is used for the diagnosis of equine influenza.

Treatment

- The antiviral drugs oseltamvir (Tamiflu), if used within 48 hours of evidence of infection, is effective in the treatment of human influenza. There is as yet no information on its use in dog flu.
- Broad spectrum antibiotics are employed in the severe form to treat secondary bacterial infections.

Prevention

- A vaccine is not yet available.

Public Health Significance

Transmission of dog flu to humans has not been reported.

Feline Influenza

In 2004, a few tigers and leopards in a Thialand zoo fed poultry carcasses infected with avian influenza (H5N1) developed a severe, fatal pneumonia as a result of H5N1 infection. Furthermore, in 2005 in the same zoo, horizontal transmission of H5N1 influenza between tigers is thought to have occurred.

It has also been reported that domestic cats can be infected experimentally (horizontally) with avian H5N1 virus. Cats thus infected developed severe diffuse alveolar damage and transmitted the virus to sentinel cats.

In 2005 an H5N1 infection was confirmed in a domestic cat in Germany; the disease was not spread to other cats. It was thought that the infection was acquired from a wild bird.

These reports suggest that the H5N1 virus should be considered a potential threat to domestic and captive felids.

It has been suggested that cross-species infection, e.g., from bird to cat requires a large infective dose of virus whereas in epidemic influenza within a species infection may result from less than ten viruses.

Glossary

Reciprocal inhibition test: A test in which inhibition titers, corresponding to the reciprocal of the last dilution that completely neutralize virus, are compared between various strains of the same virus.