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BIRD FLU: AN OVERVIEW

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Bird flu, technically known as 'Avian Influenza', has hit the country after all. After causing havoc in south-east Asian countries (Hong Kong, Vietnam, Laos, Cambodia, Indonesia, and Thailand) and the neighbouring Pakistan, bird flu finally struck India in February 2006 and again re-surfaced in January 2008 in West Bengal. In 2006, though brief, it inflicted heavy losses and jolted nation's economy. Poultry industry appeared paralyzed for a time, and fear of its spread to humans brought the industry to a virtual halt.

In 2006, the disease had suddenly flared up in the Navapur area of Maharashtra and Gujarat, Jalgoan district of Maharastra and Burhanpur district of Madhya Pradesh. Although a swift and rigorous action, jointly by the respective State Governments and the Government of India, promptly brought the disease under control, poultry industry remains vulnerable nevertheless under the constant threat of bird flu, as the virus is not to go away that easily. On the contrary, it may stay, pose a threat to industry, and perhaps risk to humans. It is on this account that the topic of bird flu has been dealt with at some length, covering its all aspects, so that the latest information is readily available. The basic theme is prevention of poultry losses, protection of the industry, and welfare of the people in view of its reportedly extremely rare human transmission.

Definition

Bird flu, is a viral disease characterized by extremely high mortality. The virus affects respiratory, digestive, and nervous system. Bird flu viruses infect, besides chicken, a wide variety of wild and domestic birds, especially the free-living birds that live in or near water, such as ducks, geese, swans, shorebirds, gulls, terns (sea birds), doves, and others. Bird flu viruses have been isolated from more than 90 species of free-living birds. Migratory waterfowl, particularly ducks, have yielded more viruses than any other group. However, most bird flu infections do not produce clinical disease in free-living birds.

History

Bird flu is not a new disease. It was discovered 130 years ago in 1878 in Italy as 'fowl plague'. It was so named because it dealt poultry a severe blow by causing heavy mortality. 'Plague' is a Latin word and means 'blow'. In 1901 its causative organism was shown to be a virus, but it was not until 1955 that its relationship to mammalian influenza A virus was demonstrated.

Cause

Bird flu virus is an enveloped, single-stranded RNA virus. Its surface is covered by two types of projections. They are glycoprotein in nature. The two projections differ in shape: (1) Haemagglutinin is a rod-shaped trimer (i.e. made up of three subunits), and (2) Neuraminidase (NA) is a mushroom-shaped tetramer (made up of four subunits). Bird flu viruses are classified on the basis of HA and NA surface antigens. At present there are 16 distinct HAs and nine distinct NAs. Each virus contains one HA and NA subtype. Thus, there can be 144 subtypes. These are identified by haemagglutinin (H) and neuraminidase (N) typing. Each type differs in its pathogenicity, capability to infect different species, and transmissibility. Although bird flu viruses can occur in numerous subtypes, the subtype H5N1 has caused most outbreaks, followed by H7N7, H7N3, H5N2, and other (given under 'Myth about H5 and H7 subtypes). Its genome (genetic material) consists of eight segments of single-stranded RNA.

The haemagglutinin is responsible for attachment of the virus to receptors present on the cell surface, and thus enables the virus to enter into the cell. On the other hand, neuraminidase, which is an enzyme, is responsible for the release of new virus from the cell by its action on neuraminic acid in the receptors. Antibodies against HA and NA are important in protective immunity. Antibodies against HA neutralize the virus and thus protect against infection. Antibodies against NA are also important in protection, by restricting the spread of the virus from infected cells.

The viral genome composed of eight segments of single-stranded RNA, codes for 10 proteins.

Chemical Composition

Bird flu viruses are composed of 0.8-1.0% RNA, 5-8% carbohydrate, 20% lipid, and 70% protein. Lipids are present in the viral envelope and are derived from the host cell.

Growth of Virus within the Cell (Viral Replication)

This process is very complex. Briefly, the virus attaches to the host cell receptors containing sialic acid bound to glycoproteins. This initiates receptor-mediated endocytosis, that is, entry of virus into the cell. In the endosomes, that is, vesicles or sacs formed, envelope of the virus fuses with the endosome membrane. The cleavage (splitting) of HA into HA1 and HA2 by proteolytic enzyme is essential for fusion and infectivity of the virus. The nucleocapsids of the virus (i.e. RNA and its surrounding protein) are transported to the nucleus where viral transcriptase complex synthesizes messenger RNA (mRNA). Transcription is initiated with 10-13 nucleotide RNA fragments. (Transcription is the mechanism by which specific information coded in a nucleic acid chain is transferred to the mRNA. This is brought about by the enzyme transcriptase). Six mRNAs are produced in the nucleus and transported to the cytoplasm for translation into HA, NA and internal proteins of the virus. (Translation is the mechanism by which a particular base sequence in the mRNA results in production of a specific amino acid sequence in the protein.) The HA and NA proteins are glycosylated (i.e. contains glycosyl radicals, derived from glucose) in the rough endoplasmic reticulum, trimmed in the Golgi and transported to the surface. Here, they are embedded in the plasma cell membrane (i.e. cell membrane). The eight viral gene segments along with internal viral proteins assemble and migrate to certain areas of the plasma membrane.

Susceptibility to Chemical and Physical Agents

Avian influenza viruses are relatively unstable in the environment. Heat, extremes of pH, and dryness can inactivate bird flu viruses. Because bird flu viruses have lipid envelopes, they are inactivated by organic solvents and detergents.

In the presence of organic matter, bird flu viruses can be destroyed by chemical inactivants such as aldehydes (formaldehyde and gluteraldehyde), and beta-propiolactone. After removal of organic matter, chemical disinfectants such as phenolics, ammonium ions (including quarternary ammonium disinfectants), oxidizing agents (such as sodium hypochlorite), dilute acids, and hydroxylamine can destroy bird flu viruses.

Laboratory versus Field Conditions

In the laboratory conditions, commonly used detergents and disinfectants (such as phenolics, quanternary ammonium surfactant compounds and sodium hypochlorite) inactivate bird flu viruses.

However, under field conditions, bird flu viruses are protected by organic material such as nasal secretions or faeces. These increase their resistance to physical and chemical inactivation. Cool and moist conditions favour long survival of bird flu viruses in the environment. Bird flu viruses have remained viable (alive) in liquid manure for 105 days in the winter and in faeces for 30-35 days at 4° C and for 7 days at 10° C.

To control field infection, it is essential to destroy the virus. This requires an integrated approach. First, heat the buildings to 90-100° F for one week. Then, remove and properly dispose of manure and litter. This is followed by cleaning and disinfection of buildings and equipment, and allowing a 2-3 weeks vacancy period before re-stocking. Virus in manure and litter must be inactivated or disposed of by burial, composting, or incineration. Effective disinfectants against bird flu viruses on clean surfaces include 5.25% sodium hypochlorite, phenolic compounds, acidified ionophor compounds, chlorine dioxide disinfectants, strong oxidizing agents, and 4% sodium carbonate / 0.1% sodium silicate. However, organic material must be removed before disinfectants can work properly.

Nature of the Disease

Bird flu viruses produce diseases that range from: (1) asymptomatic infection to (2) respiratory disease and drops in egg production to (3) severe, systemic disease with 100% mortality. This last form

of the disease results from infection by highly pathogenic bird flu viruses. Thus, based on their virulence bird flu viruses are of two types: (1) those that are of low virulence. These have been termed as 'low pathogenic' or 'mildly pathogenic avian influenza (MPAI) viruses', and (2) those that are of high virulence, capable of causing a severe disease in poultry and inflicting up to 100% mortality. These are called 'highly pathogenic or HPAI viruses'.

Although in the laboratory only two pathotypes of bird flu viruses have been demonstrated, namely, MPAI and HPAI, in the field, natural infection by bird flu virus results in a wide range of clinical diseases. This depends on virus strain, host species, and environmental factors. From the mortality patterns, symptoms and lesions, bird flu in the field occurs in four different forms: (1) Highly virulent form:- It results from infection by highly pathogenic H5 or H7 bird flu viruses in chickens (discussed later). It occurs as a severe, systemic disease that affects most organs, including brain and heart. Morbidity and mortality reach 100%. (2) Moderately virulent form:- This form results from infection by mildly pathogenic viruses, of any HA or NA subtype (discussed later), but associated with (co-infection) by secondary pathogens. The mortality rates may vary but range from 5-97% and occurs mainly in young birds, reproductively active hens, or birds under severe stress. Lesions occur in the reproductive tract, reproductive organs, kidneys, or pancreas. (3) Mildly virulent form:- This form results from infection by mildly pathogenic bird flu virus with low mortality and mild respiratory disease or drop in egg production. Mortality is usually less than 5%, typically in older birds. (4) Avirulent form:- This form results from infections by mildly pathogenic bird flu viruses. There is no mortality or symptoms of the disease. This form is the most common in wild birds with infection by mildly pathogenic bird flu viruses. In fact, disease is usually absent with bird flu virus infection in most wild bird species.

Incidence and Distribution

Bird flu viruses have a worldwide distribution. The most common source of bird flu viruses has been free-flying birds, that is, those living in or near water, especially ducks and geese, and also shorebirds, gulls, and terns (sea birds). These are considered as reservoirs of all bird flu viruses. In these species, bird flu viruses usually cause no disease (MPAI viruses), exception being high mortality in common terns of South Africa during 1961 outbreak of bird flu. Most combinations of the 16 HA and 9 NA subtypes have been reported in free-flying birds. Chickens and turkeys are not natural reservoirs of AI viruses. Most influenza infections in domestic poultry are from avian-origin bird flu viruses.

Myth about H5 and H7 Subtypes

The outbreaks of highly pathogenic bird flu between 1901 and mid-1950s involved isolates that today have been classified as H7N1 and H7N7 subtypes. However, an outbreak during 1959 in chickens in Scotland and during 1961 in Common Terns of South Africa involved H5 subtype. This led to the wrong conclusion (myth) that all H5 and H7 bird flu viruses are highly pathogenic. However, since 1971 numerous H5 and H7 mildly pathogenic bird flu viruses have been isolated and characterized. This has clarified the situation that subtypes H5 and H7 do not always mean high pathogenicity.

Antigenic Variation of Strains – Drift and Shift

Human influenza viruses have a high frequency of antigenic variation in the surface glycoproteins (HA and NA) because of two phenomena, drift and shift. This explained the antigenic change that occurred in human influenza viruses within human population. However, it is at present doubtful if such a phenomenon occurs in bird flu virus.

Antigenic drift in mammalian influenza virus arises from point mutations in the HA and/or NA genes that result in minor antigenic changes in the coding proteins.

Antigenic shift arises from genetic re-assortment between the gene segments of two influenza viruses that infect the same cell. This results in the production of new HA and/or NA antigens.

Protective Characteristic of the Virus

HA is the major antigen that produces antibodies, which protect birds against death and clinical signs. Such antibodies are HA type specific. That is, protection of the bird is HA subtype specific and

lasts for more than 35 weeks. Antibodies produced against NA provide protection against NA subtypes in birds.

Antibodies produced against internal proteins (of the virus), mainly nucleoprotein, do not protect from death or clinical signs.

Spread

Bird flu virus is excreted from the nares (nose), mouth, conjunctiva, and cloaca of infected birds into the environment. This is because virus grows in the respiratory, intestinal, renal, and/or reproductive organs.

1. The virus is spread by direct contact between infected and susceptible birds, or indirect contact through fine droplets suspended in air, or exposure to virus-contaminated fomites (inanimate objects).

2. Air spread is important because of high virus concentrations in the respiratory tract. But the virus does not appear to travel more than 45 meters through air. Therefore, airborne transmission may have a limited role in inter-flock spread of bird flu virus compared to mechanical movement of virus on equipment, clothing, or shoes. On the other hand, the large volume of low concentration of bird flu virus in infected faeces makes fomites a major means of transport.

3. Sources of infection for the first introduction of the virus into poultry include: (a) other domestic and confined poultry, (b) migratory birds (waterfowls), (c) domestic pigs, and (d) pet birds. It has been found that spread through air has a limited role as compared to mechanical movement of fomites on equipment, clothing, and shoes.

4. Introduction of bird flu viruses, especially mild pathogenic from wild birds, especially migratory waterfowl, has been documented. The source is contaminated faeces from ducks either through direct contact with poultry, or indirectly through contamination of feed or water.

5. Spread of bird flu virus during an outbreak is by mechanical transmission of virus on fomites, by air, or movement of infected poultry.

6. Whereas horizontal spread of bird flu virus commonly occurs, it does not spread vertically. However, bird flu virus infection of hens results in virus recovery from the eggshell surface and the internal contents of the eggs. Cleaning of faecal material and disinfection of egg shells may be necessary to prevent the hatchery-associated spread of AI viruses.

7. Once a flock is infected, it should be considered a potential source of virus for life.

8. Some infections of free-living perching birds, such as sparrows, have been associated with outbreaks on poultry farms where they may have acquired infections from close contact with poultry.

Role of Migratory Birds in the Spread of Bird Flu Virus

It is widely believed that migratory birds spread the bird flu infection to poultry. On the contrary, migratory birds are natural reservoirs for low pathogenic virus. In wild ducks, the viruses grow mainly in the intestinal tract, cause no signs of the disease, and are excreted in high concentrations in the faeces. This can lead to heavily contaminated pond water that could be a source of infection for other wild birds, or for poultry. Ducks occupy a very important position in the spread of bird flu virus.

Mildly pathogenic bird flu viruses are maintained in wild birds living in or near water. At times, they cross over to poultry and cause outbreaks of mild disease. Highly pathogenic bird flu viruses do not have a recognized wild bird reservoir. Highly pathogenic bird flu viruses arise from mildly pathogenic bird flu viruses through mutations in the haemagglutinin (HA) surface protein. Virulence shifts in H5 and H7 subtypes occur that facilitate the spread from wild birds to domestic poultry, resulting in highly pathogenic situations.

Migratory birds maintain mildly pathogenic bird flu viruses and do not appear to play a significant role in the spread of highly pathogenic bird flu viruses. For example, in Australia, despite the occurrence of five outbreaks of bird flu in poultry caused by H7 subtypes, there has not been a single isolate of this subtype from wild birds. The non-pathogenic subtypes isolated included H1, H3, H4, H5, H6, H11, H12, and H15.

However, in the Asian bird flu outbreaks, the presence of H5N1 viruses in dead migratory birds suggests that wild populations may be involved. While it is not known whether the H5N1 has become established in the wild bird populations, its potential role must be considered. To conclude, the extent of infection in the wild birds, their involvement in virus spread, and the range of species involved are, at present, not known.

Incubation Period

The incubation period in bird flu is 3 days in naturally-infected individual birds and up to 14 days in a flock. It depends on the dose of the virus, the route of exposure, the species exposed, and the ability to produce symptoms.

Symptoms

The symptoms are extremely variable and depend on factors such as species of the bird, sex, concurrent infections, acquired immunity, and environmental factors. However, the pathotype of the bird virus, whether it is mildly pathogenic or highly pathogenic, has the greatest impact on the clinical manifestations of the disease.

Symptoms in Mildly Pathogenic Bird Flu Viruses

In wild birds, mildly pathogenic viruses produce no symptoms.

In chickens, the most common symptoms include mild to severe respiratory symptoms. These include coughing, sneezing, abnormal respiratory sounds (rales), and excessive discharge from the eyes (lacrimation). In layers and breeders, hens may show increased broodiness and decreased egg production. In addition, they show huddling, ruffled feathers, depression, decreased activity, reduced feed and water consumption, and sometimes diarrhoea. Emaciation is uncommon because bird flu is an acute and not a chronic disease.

Symptoms of Highly Pathogenic Bird Flu Viruses

In wild birds and ducks, highly pathogenic viruses grow poorly and therefore produce almost no symptoms. The one exception is common terns in the South African outbreak of bird flu in 1961 which produced sudden deaths without showing any other symptoms.

In chickens, symptoms vary depending on the extent of damage to specific organs and tissues. That is, not all symptoms are present in every bird. In most cases, the disease attacks suddenly and is extremely severe. Some birds are found dead before any symptoms are seen. If the disease is less severe and birds survive for 3-7 days, individual birds may show nervous disorders, such as tremors of head and neck, inability to stand, twisting of the neck, and unusual positions of head and legs. The poultry houses are usually quiet because of decreased activity and reduction in normal vocal sounds of the bird. Depression and decrease in feed and water consumption are common. Sudden drop in egg production occur in breeders and layers. The drop in egg production go on increasing, and within six days there is total stoppage of egg production.

Respiratory symptoms are less common than with mildly pathogenic viruses, but can include abnormal respiratory sounds, sneezing, and coughing.

Morbidity and Mortality

In chickens, morbidity and mortality rates are as variable as the symptoms. These depend on virulence of the virus and the host as well as age, environment, and concurrent infections. For mildly pathogenic viruses, high morbidity and low mortality rates are typical. Mortality is usually less than 5%, unless accompanied by secondary pathogens, or if the disease is in young birds. With the highly pathogenic viruses, morbidity and mortality rates are very high (50-90%) and can reach 100% in certain flocks.

In wild birds, mildly pathogenic viruses usually produce no disease or deaths.

Postmortem Findings

Postmortem findings are extremely variable, depending on the pathogenicity of the infecting virus, and presence of secondary pathogens.

In Mildly Pathogenic Bird Flu Viruses

In poultry, the most common lesions are in the respiratory tract, especially sinuses, and are characterized by different types of inflammation. The tracheal mucosa can be swollen (oedematous) with congestion and sometimes haemorrhages. Tracheal exudates may vary from serous to caseous with, at

times, blockage of airways resulting in asphyxiation (suffocation). Airsacculitis may be present. The inflammation is usually associated with secondary bacterial infections. The infraorbital sinuses may be swollen and mucous to mucopurulent nasal discharge occurs. Bronchopneumonia can result when secondary pathogens such as Escherichia coli and Pasteurella multocida flare up.

In the body cavity, there may be catarrhal to fibrinous peritonitis, and even egg yolk peritonitis may be observed. Enteritis may be observed in the caeca and/or intestine. Inflammatory exudates may be .found in the oviduct of laying birds, and the last few eggs laid have reduced calcium deposition in the eggshells. Rarely eggs are misshapen and fragile with loss of pigmentation. Ovaries undergo regression, beginning with haemorrhage in the large follicles and progressing to colliquation. That is, the ovary becomes softened and liquefied. The oviduct may be oedematous and contain exudates before undergoing involution (shrinkage). In a few cases in laying hens, kidneys may be swollen and accompanied by visceral gout.

In Highly Pathogenic Bird Flu Viruses

In poultry, highly pathogenic bird flu viruses produce a variety of oedematous, haemorrhagic, and necrotic lesions in internal organs and the skin. However, if the death is sudden, no gross lesions may be seen. In chickens, swelling of the head, face, upper neck and feet are common as a result of subcutaneous oedema. The eyes may show excessive discharge, and swelling around the eye is common. These changes are accompanied by small to large haemorrhages below the skin in the feet. Necrotic foci, haemorrhage, and cyanosis of the non-feathered skin is common, especially wattles, combs, and legs.

Lesions in the internal organs vary with virus strain, but are mostly haemorrhages on serosal and mucosal surfaces and foci of necrosis inside the internal organs. Most common are prominent haemorrhages on the surface of the heart (epicardium), in the breast and leg muscles, and in mucosa of the proventriculus and gizzard and, at times, small intestine. Necrotic foci are common in pancreas, spleen, and heart, and sometimes in liver and kidney. Trachea may be highly congested and in severe cases may exhibit severe haemorrhages. This was particularly seen in the Indian outbreak. Lungs show pneumonia with oedema. The lungs can be congested or haemorrhagic.

Note: The postmortem findings described, at best, only suggest that the disease may be bird flu. They are by no means specific, since such findings can also be seen in certain other diseases of poultry. Definitive diagnosis is established only by isolation and identification of the bird flu virus.

Development of the Disease

The disease begins by inhalation or ingestion of the bird flu viruses. In poultry, the nasal cavity is a major site of initial growth (replication).

With highly pathogenic viruses, the virions invade the submocosa of the respiratory or intestinal tract, and enter into capillaries. The virus replicates (grows) within endothelial cells of these vessels and spreads through the vascular or lymphatic systems to infect and grow in a variety of different cells in internal organs, brain, and skin. It may also happen that it may spread to different organs, before its extensive growth in the endothelial cells of the vessels. Symptoms and death are due to multiple organ failure. Damage caused by bird flu viruses is the result of any one of these three processes: (1) direct virus growth in cells, tissues, and organs. (2) indirect effects from production of cellular mediators such as cytokines, and (3) ischaemia (inadequate flow of blood) from vascular thrombosis.

With mildly pathogenic viruses, replication (growth) is usually limited to the respiratory or intestinal tract. Like highly pathogenic viruses, it does not spread to internal organs. Illness or death is usually from respiratory damage, especially if accompanied by secondary bacterial infections. Sometimes, the mildly pathogenic viruses spread systemically, replicating and causing damage in kidney, pancreas, or other organs.

Immunity

Active: Infection with bird flu viruses as well as immunization with vaccine produces a humoral antibody response at both systemic and mucosal levels. This includes systemic IgM response five days

after infection, followed soon by an IgG response. The mucosal immune response is poorly characterized.

Antibodies against the surface proteins of the virus (HA and NA) are neutralizing and protective. Protection is mainly associated with antibodies detected against the HA protein. However, either HA, NA, or both prevent clinical signs and death. Duration of protection is unknown, but in layers, protection against symptoms and death has been shown to be at least for 30 weeks following a single immunization. Birds that have recovered from field exposure are protected from the same HA and NA subtypes.

Immune response against internal proteins does not prevent symptoms and death, but may shorten the period of virus replication and shedding. However, the mechanism of this limited protection is unknown, but may be the result of cell-mediated immunity.

Passive: Not much is known on the protective role of maternal antibodies. But based on evidence available for other diseases, protection against symptoms and death from the bird flu virus is likely for the first two weeks after hatching.

Diagnosis

A definitive diagnosis is established by: (1) Direct detection of bird flu viral proteins or genes in specimens such as tissues, swabs, cell cultures, or embryonating eggs, or (2) Isolation and identification of bird flu virus. A presumptive diagnosis can be made by detecting antibodies to bird flu virus.

Sample Selection and Storage

1. Bird flu viruses are usually recovered from tracheal or cloacal swabs of either live or dead birds. This is because most highly and mildly pathogenic viruses replicate in the respiratory and intestinal tracts.

2. The swabs should be placed in sterile transport medium containing high levels of antibiotics to reduce bacterial growth.

3. Tissues, secretions, or excretions from these tracts are appropriate for virus isolation.

4. Tissues can be collected and placed into sterile plastic tubes or bags.

5. In the examination of organs for virus, collect and store internal organs separately from the respiratory and intestinal tract tissues because isolation of virus from internal organs may be an indication of systemic spread and is usually associated with highly pathogenic viruses. In case of systemic infections produced by highly pathogenic viruses, almost every organ can yield virus because of the high levels of viraemia or replication in parenchymal cells.

6. If the samples can be tested within 48 hours, they may be kept at 4° C. However, if the sample is to be held for a longer time, storage at -70° C is recommended.

7. Before testing for virus, tissues should be ground as a 5-10% suspension in the transport medium and clarified by low-speed centrifugation.

Direct Detection of Bird Flu Viral Proteins of Nucleic Acids: The direct demonstration of bird flu virus RNA or viral proteins in samples from birds is not routinely used for diagnosis at this time.

Virus Isolation: Chicken embryos, 10-11 days-old, are inoculated through the allantoic cavity with about 0.2 ml of sample.

The death of inoculated embryos within 24 hours after inoculation usually results from bacterial contamination or inoculation injury. These eggs should be discarded. A few viruses may grow rapidly and kill the embryos by 48 hours. However, in most cases the embryos will not die before this time. After 72 hours, or at death, the eggs should be removed from the incubator, chilled, and allantoic fluids should be collected. The presence of virus is demonstrated by chicken erythrocyte haemagglutinating activity in the allantoic fluid.

Generally, if a virus is present in a sample, there will be enough growth in the passage to result in haemagglutination, and repeated passage is unnecessary. Repeated passage of samples increases the risk of cross-contamination in the laboratory.

Long-term storage of virus should be done at -70° C.

Virus Identification

1. Standard methods for testing the egg fluids for the presence of haemagglutinating activity (antibodies) are through the use of chicken erythrocytes by macro- or micro-techniques. Allantoic fluid positive for haemagglutination is used for virus identification.

2. It is important to find out whether the haemagglutinating activity detected in the allantoic fluid is due to influenza virus, or other viruses such as Ranikhet disease virus. Therefore, the isolate is tested in HI assays against Ranikhet disease and other antiserum.

3. If negative, the virus is then tested for the presence of the type A specific antigen to confirm that an influenza A virus is present.

4. The next step in the identification is to determine the antigenic subtype of the surface antigens HA and NA. The NA subtype is identified by a micro-NI assay with antisera prepared against the 9 known NAs. This NI assay is usually the first assay that is done on an isolate.

5. The HA is identified in the HI test using a panel of antisera prepared against the 16 distinct HAs.

6. The final identification is done by the Government of India's High Security Laboratory located at Bhopal, Madhya Pradesh, India.

Serology:

1. Serological tests are used to detect the presence of bird flu virus-specific antibodies as early as 7 days after infection.

2. Several techniques are used for serological surveillance and diagnosis. For surveillance, a double immunodiffusion test for the detection of anti-NP antibody is generally used. This is because it detects antibodies to type A specific antigens shared by all influenza A viruses.

3. ELISA assays (tests) have been developed to detect antibody to influenza. Once influenza is detected by immunodiffusion or ELISA, HI tests can be used to determine HA subtype.

Differential Diagnosis

1. Infections that must be considered in the differential diagnosis include Ranikhet disease, infectious bronchitis, infectious laryngotracheitis, and mycoplasmosis.

2. Concurrent infections with other viruses or other bacteria are commonly observed.

Treatment

There is no satisfactory treatment. Supportive care and treatment with broad-spectrum antibiotics may reduce the effects of concurrent bacterial infections.

Prevention and Control

The basic approach in the control of bird flu is preventing the first introduction of the virus and controlling the spread once it is introduced. One critical aspect in the prevention and control is the education of poultry industry regarding how the viruses are introduced, how they spread, and how such events can be prevented.

Prevention

1. The most important source of virus for poultry is other infected birds. Therefore, the first step towards prevention of infection is the separation of susceptible birds from infected birds and their secretions and excretions. Biosecurity is the first line of defence.

2. Spread can occur when susceptible and infected birds are in close contact, or when infectious materials from infected birds are introduced into the environment of susceptible birds. Such outbreaks occur with the movement of equipment, footwear (shoes, etc.) and clothing, vehicles, insemination equipment, feed, water, etc. The presence of virus in faecal material is the source of virus for its movement through equipment and people.

3. There should be no contact with recovered flocks, because the length of time for which birds within a population shed virus is not clearly known.

4. Wild birds are the reservoir of influenza viruses. They should be considered a major source of infection for poultry. Therefore, it is important to reduce the contact between these two groups.

Control

1. Bird flu virus is excreted from both respira-

tory and digestive systems. Therefore, within a poultry house, bird-to-bird transmission is by air and ingestion. Contaminated poultry faeces are the most important source of spread between flocks.

2. After the bird flu virus has been introduced into commercial flocks, certain things must be identified that contribute to its spread. These include unclean equipment and people, marketing an actively infected flock, and inadequate cleaning and disinfection.

3. All methods for controlling the spread of bird flu are based on preventing contamination and controlling the movement of people and equipment. Persons who have direct contact with birds or their faeces have been the cause of most disease spread between houses or premises. Equipment that comes in direct contact with birds or their faeces should not be moved from farm to farm without adequate cleaning and disinfection. Also, it is important to monitor that the traffic area near the poultry house does not get contaminated with faeces.

4. Even before the occurrence of bird flu, vigorous control measures must already be in operation. If the virus turns out to be highly pathogenic, it could take a few weeks from initial sickness until a government emergency can be declared. Therefore, voluntary efforts of poultry industry/ farmers to control the initial outbreak are most important.

5. The farm-to-farm spread of bird flu virus must first be brought under control before the disease can be eradicated.

6. Most of the bird flu virus shed from an infected flock occurs during the first two weeks of infection. Serologically positive flocks are not associated with a high risk of spread. Usually by four weeks after the infection, virus cannot be detected.

7. In the case of mildly pathogenic outbreaks, efforts must focus on preventing spread of the disease beyond the first case. In the past, outbreaks in USA, Mexico, and Italy have shown that highly pathogenic virus can emerge from mildly pathogenic outbreaks. In these cases, highly pathogenic bird flu virus emerged from mildly pathogenic H5 or H7 viruses circulated widely in susceptible poultry flocks for several months. In contrast, 20 mildly pathogenic outbreaks of H5 or H7 eliminated within three months in USA did not result in the emergence of highly pathogenic bird flu virus. This illustrates the need for prompt responses against mildly pathogenic outbreaks. Prevention and control of mild bird flu outbreaks are the most important steps to prevent outbreaks of highly pathogenic virus.

8. With highly pathogenic virus, eradication procedures (quarantine, slaughter, disposal, and clean-up) must be employed. Quarantine of the infected area is essential to prevent spread and to eradicate the disease.

Vaccination

1. Killed bird flu virus vaccines have been used. Their effectiveness in preventing symptoms and mortality are well documented. However, protection is virus subtype specific. Birds are susceptible to infection with influenza viruses belonging to any of the 16 HA subtypes. Moreover, there is no way to predict their exposure to any particular one. It is not practical to use preventive vaccination against all possible subtypes. However, after an outbreak occurs and the subtype of the virus is identified, vaccination may be a useful tool.

2. Killed monovalent and polyvalent virus vaccines, with adjuvants, are capable of producing antibody and providing protection against mortality, morbidity, and drop in egg production.

3. Controlled use of vaccines in a mildly pathogenic H5 or H7 outbreak may delay and reduce the chance of the emergence of highly pathogenic viruses. However, their use is still debated.

4. Vaccinated flocks cannot be considered bird flu virus-free. However, use of vaccine reduces the amount of virus shed in experimentally vaccinated and challenged birds. This reduces shedding and potential spread of the virus to other birds.

5. Controlled, effective use of vaccine will reduce the population of susceptible poultry and reduce the quantity of virus shed if infection occurs.

6. To conclude, because of the large number of influenza A viruses in migratory and wild bird populations, the viruses will continue to cause serious disease problems in the commercial poultry industry. Judicious use of vaccines may therefore be appropriate to reduce spread and decrease suscep-

tibility of poultry to the virus. This would enable implementation of eradication methods before the disease spreads and becomes endemic.

References

1. Alexander, D. J. 1982. Avian influenza. Recent developments. Vet. Bull. 52: 341-359

2. Alexander, D. J. 1995. The epidemiology and control of avian influenza and Newcastle disease. J. Comp. Pathol. 112: 105-126

3. Alexander, D. J. 2000. A review of avian influenza in different bird species. Vet. Microbiol. 74: 3-13

4. Alexander, D. J. 2000. The history of avian influenza in poultry. World Poultry 7- 8

5. Capua, I. and S. Marangon. 2000. The avian influenza epidemic in Italy, 1999-2000: A review. Avian Pathol 29: 289-294

6. Dybing, J.K., S. Schultz Cherry, D.E. Swayne, D.L,Suarez, and M.L. Perdue. 2000. Distinct pathogenesis of Hong Kong-origin H5N1 viruses in mice as compared to other highly pathogenic H5 avian influenza viruses. J. Virol. 74: 1443-1450

7. Halvorson, D.A. 1998. Strengths and weaknesses of vaccines as a control tool. In D.E. Swayne and R.D. Slemons (eds.). Proceedings of the Fourth International Symposium in Avian Influenza. U. S. Aninmal Health Association: Richmond, VA, 223-227

8. Horimoto, T., E. Rivera, J. Pearson, D. Senne, S. Krauss, Y. Kawaoka, and R.G. Webster. 1995. Origin and molecular changes associated with emergence of a highly pathogenic H5N2influenza virus in Mexico. Virology213: 223-230

9. Morgan, I.R. and A.P. Kelly. 1990. Epidemiology of an avian influenza outbreak in Victoria in 1985. Aust. Vet. J. 67: 125-128

10. Mounts, A.W., H. Kwong, H.S. Izurieta, Y. Ho, T. Au, M. Lee, B.C. Buxton, S.W. Williams, K.H. Mak, J.M. Katz, W.W. Thompson, N.J. Cox, and K. Fukuda. 1999. Case-control study of risk factors for avian influenza A (H5N1) disease, Hong Kong, 1997. J. Infect. Dis, 180: 505-508

11. Perdue, M. L. and D. L. Suarez2000. Structural features of the avian influenza virus haemagglutinin that influence virulence. Vet. Microbiol. 74: 77-86

12. Perdue, M.L., D.L. Suarez, and D. E. Swayne. 1999. Avian influenza in the 1990s. Poultry and Avian Biological Reviews 11: 1-20

13. Seo, S.H. and R.G. Webster. 2001. Cross-reactive, cell-mediated immunity and protection of chickens from lethat H5N1 influenza virus infection in Hong Kong Poultry markets. J. Virol. 75: 2516-2525

14. Shortridge, K. F. 1999. Poultry and the influenza H5N1 outbreak in Hong Kong, 1977: Abridged chronology and virus isolation. Vaccine 17: S26-S29

15. Suarez, D.L., M. L. Perdue, N. Cox, T. Rowe, C. Bender, J. Huang, and D. E. Swayne. 1998. Comparison of highly virulent H5N1 influenza A viruses isolated from humans and chicken from Hong Kong. J. Virol. 72: 6678-6688

16. Suarez, D. L. and C.S. Schultz. 2000. Immunology of avian influenza virus: A review. Dev. Comp. Immunol. 24: 269-283

17. Swayne, D. E. and D. A. Halvorson. 2003. Influenza. In Poultry Diseases. Edited by Y.M. Saif. 11th Edition. Pp. 135-160. Published by Iowa State Press, 2121 State Avenue, Ames, Iowa 50014, USA

18. Swayne, D. E. and R. D. Slemons. 1990. Renal pathology in specific-pathogen-free chickens inoculated with a water-fowl-origin type A influenza virus. Avian Dis 34: 285-294

19. Swayne, D. E. and D. L. Suarez. 2000. Highly pathogenic avian influenza. Rev. Sci. Tech. Off. Int. Epiz. 19: 463-482

20. Vegad, J. L. 2008. Poultry Diseases. A Guide for Farmers and Poultry Professionals. Second Revised and Enlarged Edition. Published by International Book Distributing Co., Khushnuma Complex, Basement,7, Meerabai Marg,(Behind Jawahar Bhawan), Lucknow-226001, U.P. pp. 42- 64

21. Webster, R. G. W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. 1992. Evolution and ecology of influenza A viruses. Microbiol. Rev.

56: 152-179

22. Webster, R. G., Y. Kawaoka, W. J. Bean, C. W. Beard, and M. Brugh. 1985. Chemotherapy and vaccination. A possible strategy for the control of highly virulent influenza virus. J. Virol. 55: 173-176

23. Webster, R. G. and R. Rott. 1987. Influenza virus A pathogenicity: The pivotal role of haemagglutinin. Cell 50: 665-666

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