Avian Viral Diseases

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Avian Influenza

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Avian influenza (AI) is caused by type A influenza virus classified into 16 hemagglutinin (H1–H16) and nine neuraminidase (N1–N9) subtypes.

- **Public Health Significance**
- **Etiology**
- **Pathobiology**
- **Diagnosis**
- **Intervention Strategies**

Public Health Significance

■Aquatic birds are the natural hosts of Type A influenza viruses.

➢Spillover of viruses from wild aquatic birds to poultry and other species occurs frequently.

■AI viruses or their genes have been transferred to humans.

➢Transfer of complete AI viruses (in toto) with individual sporadic infections; or appearance of individual AI viral gene segments in pandemic human influenza viruses.

➢**The H5Nx Gs/GD lineage HPAI viruses, and H7N9 LPAI and HPAI viruses have caused more clinically significant human infections than all the other AI viruses combined.**

AIV transmission and Carriers

Wild bird migration

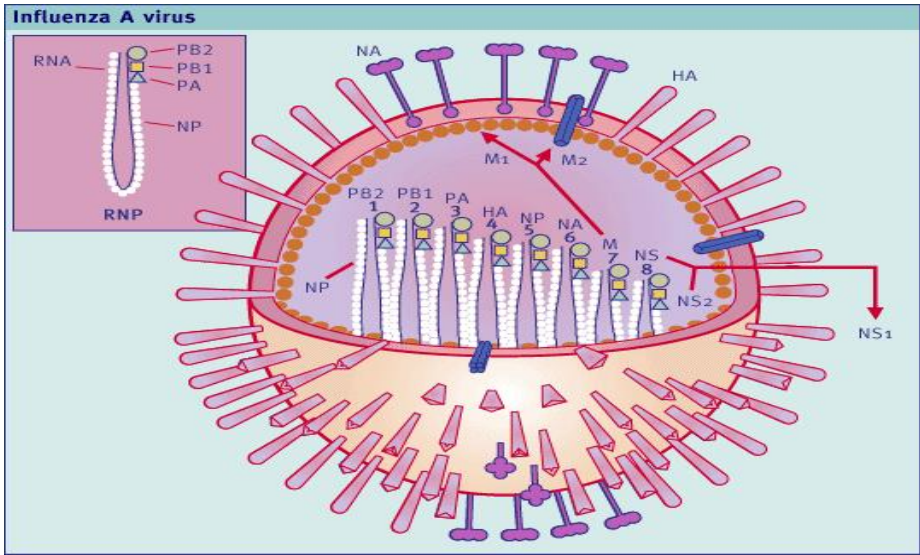
Development of a Highly Pathogenic Strain and fecal transmission

Fecal and Waterborne

Airborne and Fecal Waterborne

Etiology

- **Avian influenza viruses are classified in the family Orthomyxoviridae, genus Influenza virus A.**
- **The surface is covered by two types of glycoprotein projections.**
  - Rod-shaped trimers of hemagglutinin (HA)
  - Mushroom-shaped tetramers of neuraminidase (NA)
- **The viral genome is composed of eight segments of single-stranded, negative sense RNA that code for a minimum of 10 or up to 17 proteins.**
  - Eight proteins are constituents of the virus (HA, NA, nucleoprotein [NP], matrix 1 [M1], matrix 2 [M2], polymerase basic protein 1 [PB1], polymerase basic protein 2 [PB2], polymerase acidic protein [PA]).



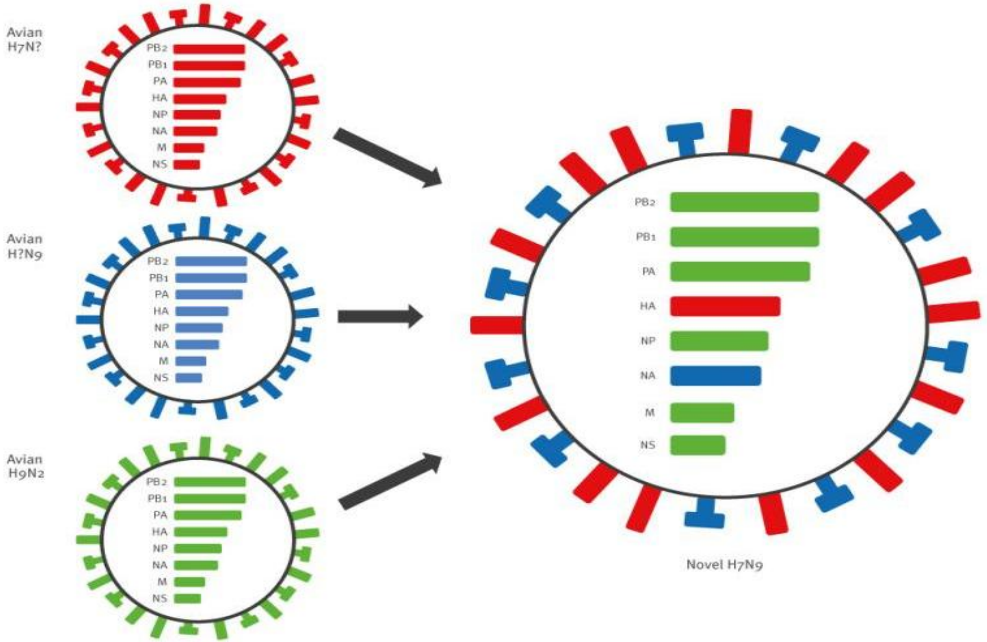
Strain Nomenclature

- The naming of the influenza virus strains includes the genus or type (A, B, C, or D), host of origin (except for human where the "host of origin" is omitted), geographic site, strain number (if any), and year of isolation followed by the antigenic subtype designating HA (H) and NA (N) in parentheses for type A influenza viruses. For example, "A/turkey/Minnesota/12582/2015 (H5N2)".
- Sixteen subtypes of HA (H1–16) and nine subtypes of NA (N1–9) are recognized for AI viruses. Serologic subtyping of HA is done by the HI test and subtyping of NA by neuraminidase inhibition (NI) test or gene sequencing.
- Based on HA gene sequences, H5Nx Gs/GD lineage HPAI viruses have been further classified into first order clades (e.g., 0–9) and some second (e.g., 2.2), third (e.g., 2.2.1), fourth (e.g., 2.3.2.1), and fifth (e.g., 2.3.2.1c) order clades to reflect molecular variation and evolution in genes encoding the HA protein.

Antigenic Variation of Strains

Drift -- Antigenic drift in influenza viruses arises from point mutations in the HA and/or NA genes that results in antigenic changes in the coding proteins.

Shift -- Antigenic shift arises from reassortment between influenza gene segments coding the surface proteins, most importantly the HA.



Pathotype

highly pathogenic avian influenza (HPAI)

- **HPAI viruses have an intravenous pathogenicity index (IVPI) in six-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in four- to eight-week-old chickens infected intravenously.**

low pathogenicity avian influenza (LPAI)

- **LPAI viruses are all influenza A viruses of H5 and H7 subtypes that are not HPAI viruses.**

The non-H5 and non-H7 LPAI viruses, for which there is no formal equirement to report to OIE unless they are causing a severe disease, still may be reportable to national and state/provincial authorities.

Laboratory Host Systems

Embryonating chicken eggs

- The preferred method for isolation and propagation of AI viruses has been 9–11- day- old embryonating chicken eggs inoculated via the chorioallantoic sac (CAS).

Cell culture systems

- Primary cultures of chicken embryo fibroblasts (CEF) or kidney cells are most commonly used for plaque assays and virus neutralization tests. Madin–Darby canine kidney (MDCK) cell cultures have also been used.

Animals

- The chicken has been the most frequently used animal in laboratory studies to determine pathogenicity and study pathogenesis. Other commonly used laboratory species include the turkey, domestic duck, house mouse, guinea pig, and ferret. The mouse, guinea pig, and ferret have been used as models to assess the risk of interspecies transmission of AI viruses to humans.

Clinical Signs

HPAI

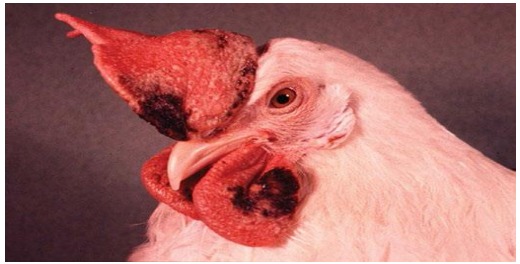
- In wild and domestic waterfowl, most HPAI viruses replicate to a limited degree and produce few clinical signs.
- In most cases in chickens and turkeys, the disease is fulminating with some birds being found dead prior to observation of any clinical signs.
- Individual birds may exhibit nervous disorders such as tremors of the head and neck, inability to stand, torticollis, opisthotonus, and other unusual positions of head and appendages.
- With the HPAI viruses, morbidity and mortality rates are high (50–89%) and can reach 100% in some flocks.

Clinical Signs

LPAI

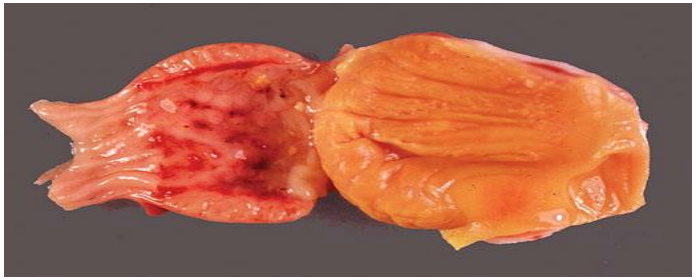
- Most infections by LPAI viruses in wild birds produce no clinical signs.
- The most frequent signs represent infection of the respiratory tract and include mild to severe respiratory signs such as coughing, sneezing, rales, rattles, and excessive lacrimation. In layers and breeders, hens may exhibit increased broodiness and decreased egg production.
- generalized clinical signs including huddling, ruffled feathers, listlessness, decreased activity, lethargy, decreased feed and water consumption, and occasionally diarrhea.
- For the LPAI viruses, high morbidity and low mortality rates are typical. Mortality rates are usually less than 5% unless accompanied by secondary pathogens or if the disease is in young birds.

Pathology

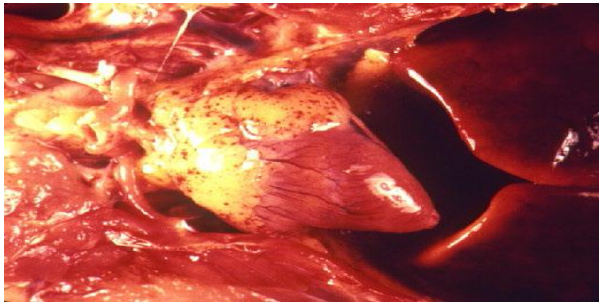


Necrosis and hemorrhage of comb and wattles

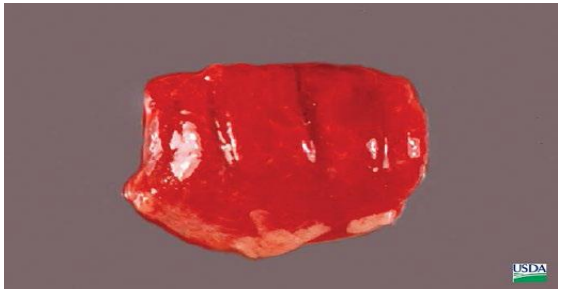
Severe subcutaneous hemorrhages of feet



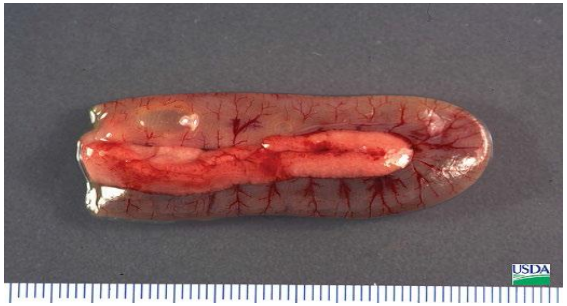
Mucosal petechial hemorrhages surrounding proventricular glands



Petechial hemorrhages in epicardial fat



Severe pulmonary hemorrhage and edema



Hemorrhage and necrosis in the pancreas

Diagnosis

- **A definitive diagnosis of AI is established by:**
  - **direct detection of AI viral proteins or nucleic acid in specimens such as tissues, swabs, cell cultures, or embryonating eggs.**
  - **isolation and identification of AI virus.**
- **A presumptive diagnosis can be made by detecting antibodies to AI virus.**
  - **During outbreaks of HPAI, mortality rates, clinical signs, and lesions may be useful as part of the case definition.**

Diagnosis

Sample Selection and Storage

- Avian influenza viruses are commonly recovered from tracheal, oropharyngeal, or cloacal swabs of either live or dead birds.
- Tissues, secretions, or excretions from these tracts are appropriate for virus isolation or detection.

Direct Detection of AI Viral Proteins or Nucleic Acids

- The direct demonstration of influenza virus RNA or viral proteins in samples from animals is routinely used as a diagnostic screening test.
- Reverse transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR (rRT-PCR) methods are routinely used for field case diagnosis of AI. The rRT-PCR has a three-hour test time, and sensitivity and specificity comparable to virus isolation procedures.
- Screening of tracheal or oropharyngeal samples from birds are done using a matrix gene rRT-PCR test and if positive, the samples are reflexively tested with H5 and H7 subtype specific rRT-PCR tests.

Diagnosis

Virus Isolation

- Chicken embryos, 9–11 days old, are inoculated via the allantoic cavity with approximately 0.2 mL of sample.
- The presence of virus is demonstrated by hemagglutinating activity.

Virus Identification

- The isolate is tested in HI assays against Newcastle disease and other antiserum. If negative, the virus then is tested for the presence of the type A specific antigen to establish that an influenza A virus is present.
- The next step in the identification procedure is to determine the antigenic subtype of the surface antigens, HA and NA.
- The direct sequencing of influenza viruses is becoming more common which allows for direct HA and NA subtyping.

Serology

- Serologic tests are used to demonstrate the presence of AI-specific antibodies. In general the ELISA tests are more sensitive than AGID or HI.

Intervention Strategies

Management Procedures

- Interventions for AI include measures designed to: (1) prevent introduction of virus, (2) reduce the likelihood of infection of birds once virus is introduced, (3) prevent movement of virus from a premise with infected birds to another premise and, if possible, (4) eliminate/eradicate the virus.

- These outcomes are accomplished using combinations of five specific, interrelated components: (1) biosecurity (including modifications to the way poultry are reared and sold, movement management, and cleaning and disinfection), (2) active and passive surveillance (and associated diagnostic services), (3) elimination of infection in poultry (mainly through stamping out), (4) decreasing host susceptibility (mainly through vaccination), and (5) education including risk communication.

Vaccination

Types of Vaccine

- The most frequently licensed AI vaccine technology has been inactivated whole AI virus adjuvanted vaccines, typically made using LPAI field outbreak strains or reverse genetic generated AI vaccine strains, followed by chemical inactivation and oil emulsification.
- Live recombinant fowl poxvirus, herpesvirus of turkeys, and Newcastle disease vaccines with AI H5 gene inserts (rFPV-AIV-H5, rHVTAIV-H5, and rNDV-AIV-H5, respectively) have been licensed and are used in a few countries.

Immunization Procedure

- In general the live vaccine is used primarily as a priming vaccine at one day of age followed by a boost 10–21 days later with inactivated AI vaccine.

Avian Viral Diseases

Newcastle Disease

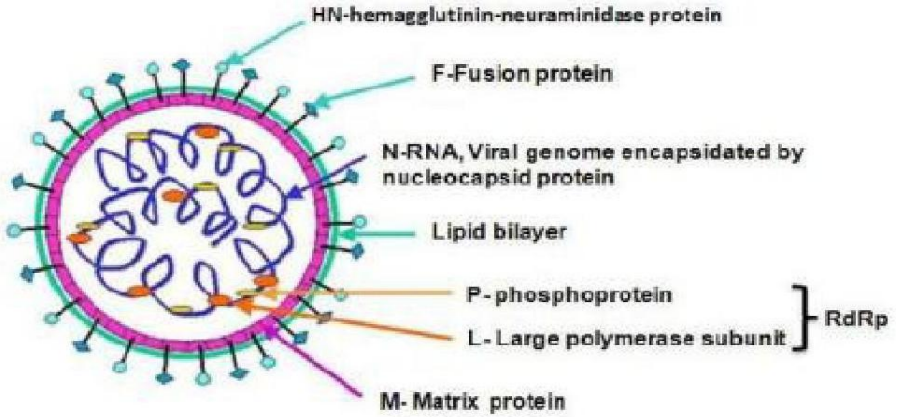
Newcastle Disease

Virulent strains of avian paramyxovirus 1 (APMV-1), from the genus Orthoavulavirus and species avian orthoavulavirus 1, infect at least 236 species of wild birds and poultry species leading to Newcastle disease.

- **Etiology**
- **Pathobiology**
- **Diagnosis**
- **Intervention Strategies**

Etiology

- NDV or APMV-1 is classified in the family Paramyxoviridae, genus Orthoavulavirus and species avian orthoavulavirus 1.
- The fusion (F) and hemagglutinin-neuraminidase (HN) glycoproteins, 17 nm in length, are densely packed onto the surface of the virion.
- The NDV genome is composed of six structural proteins, listed from 3′ to 5′: nucleocapsid protein (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and the RNA-dependent RNA polymerase (RNAP), designated the large polymerase (L).



Pathotype

Velogenic

- Viscerotropic velogenic NDV (vvNDV), consisting of hemorrhagic lesions in the gastrointestinal tract and often neurologic signs;
- neurotropic velogenic NDV (nvNDV) produces neurological signs with some respiratory involvement.

Mesogenic

- Mesogens are less pathogenic, producing neurological disease with deaths in only young birds.

Lentogenic

- Lentogens cause primarily respiratory infections that in specific pathogen free (SPF) chickens cause asymptomatic infections, and are commonly used as live ND vaccines.

Asymptomatic enteric

- Viruses of the asymptomatic enteric pathotype have a tropism for the gastrointestinal tract, and are not thought to cause or contribute to clinical disease and are also used as live vaccines.

Pathogenicity Tests

- The terms “velogenic,” “mesogenic,” and “lentogenic” were defined as the mean death times (MDT) in chicken embryos, after allantoic sac inoculation, of less than 60 hours, 60–90 hours, and greater than 90 hours, respectively.
- The ICPI in day-old chicks differentiates lentogenic viruses with ICPI values of less than 0.7 from virulent mesogenic strains with ICPI values equal to or greater than 0.7 and less than 1.5, and velogenic viruses with ICPI values greater than 1.5.

Virulence Determinants

- The amino acid sequence of the F0 precursor of loNDV are characterized by a monobasic amino acid sequence motif at the C-terminus of the F2 protein and a leucine at the N-terminus of the F1 protein, ^{112}G-R/K-Q-G-R↓L^{117}.
- Virulent strains have a multibasic amino acid sequence motif at the C-terminus of the F2 protein, ^{112}R/G/K- R- Q/K- K/R- R↓F^{117}, and a phenylalanine at the N-terminus of the F1 protein.

Laboratory Host Systems

Embryonating chicken eggs

- Most avian paramyxoviruses replicate to high titers with allantoic sac inoculation into SPF embryonating chicken eggs (ECE).

Cell culture systems

- The most commonly used cells are primary chicken embryo fibroblasts (CEF) and kidney cells (CEK), chicken fibroblast cell line (DF1), African green monkey kidney epithelial cell line (VERO), baby hamster kidney fibroblast cell line (BHK), and human epithelial type-2 cell line (Hep-2).

Animals

- In addition to poultry species, pigeons, cormorants, and psittacines are commonly infected with vNDV.

Clinical Signs

Nonvaccinated chickens infected with vvNDV

- Become listless and depressed two days after infection, ending with 100% mortality by the third or fourth day;
- With an oculonasal route of infection, bilateral conjunctivitis with some facial swelling may be present. Often clear mucus will pour from the mouths of infected birds if their heads droop toward the ground, leaving the bird gasping for air as it tries to clear the oral cavity of fluid.

Nonvaccinated chickens infected with nvNDV

- Birds may seem excitable and hypermetric three or four days after infection. Approximately five days after infection, head or muscular tremors, torticollis, and paralysis of one wing or one leg may occur.
- Mortality is usually around 50%, with greater mortality in younger birds.

Clinical Signs

Well-vaccinated layers infected with vNDV

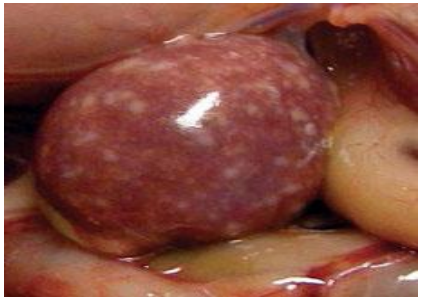
- Only a decrease in egg production one week after infection;
- At one month after infection, misshapen and/or bleached eggs may appear for the life of the chicken
- Strains of low virulence usually do not cause disease in adult birds. However, young birds may present with serious respiratory distress.

Pathology

Birds appear depressed

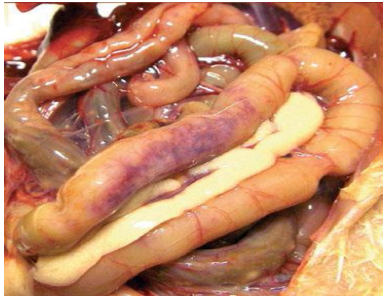
Misshapen eggs

Enlarged, mottled spleen

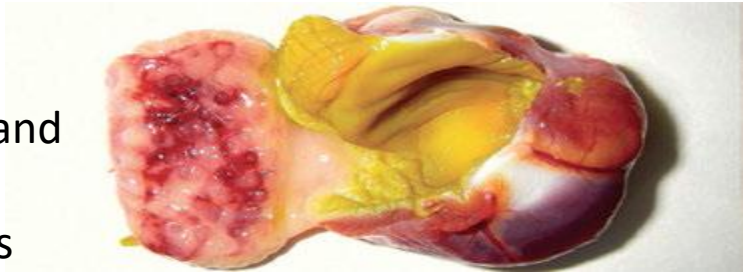


Necrosis and hemorrhage of the cecal tonsils

Necrosis and hemorrhage of small intestinal lymphoid patches



Hemorrhage and necrosis of proventriculus



Diagnosis

Isolation and Identification of NDV

- Tracheal, oropharyngeal, and/or cloacal swabs from live birds, or organ tissue samples (brain, liver, spleen, kidney, or organs with lesions) from dead birds suspended in an antibiotic/antifungal media should be kept at 4 °C for 1–2 hours or frozen. The processed samples are inoculated into the chorioallantoic sac of a 9- to 11-day-old SPF ECE and examined daily.
- Allantoic fluid from any eggs containing embryos that died after 24 hours and all eggs at the end of the 7- day incubation period should be collected after the eggs are chilled at 4 °C to be tested for hemagglutinating activity (HA). All HA-positive samples should be tested for specific inhibition with an antiserum to NDV (APMV-1), referred to as a hemagglutination-inhibition (HI) assay. All HA-negative samples should be passaged again in ECE and tested for HA activity.

Diagnosis

Serology

- Serology usually is not a useful tool for the diagnosis of ND, because current serologic methods cannot differentiate antibodies induced from an infection with vNDV, loNDV from wild birds, or those induced by vaccination with live or inactivated vaccines.
- Diagnostically, serology is most often used to measure the effectiveness of a vaccination program.
- Hemagglutination-inhibition (HI) assays and enzyme-linked immunosorbent assays (ELISAs) are used most commonly to detect and quantify antibodies to NDV.

Molecular Techniques

- Conventional RT-PCR
- Real-time RT-PCR （RRT-PCR）
- multiplex RRT-PCR

Intervention Strategies

Vaccination

- The role of vaccination in the control of ND has been to prevent losses from morbidity and mortality.
- Vaccination may increase the resistance to infection and reduce the quantity of vNDV shed resulting at the end in fewer birds being infected.

Field Vaccination Protocols and Regimens

- In general, broiler breeders receive at least three live vaccines over their life span and broilers get one live vaccine at day of age with one or two live booster vaccines later in the field; Layers will likely receive one inactivated and three live vaccines, with additional live vaccine boosts, over their life span.
- The protective immune response is usually evaluated by examination of HI antibody titers. An HI titer equal or greater than 16 is considered positive.

Intervention Strategies

Types of Vaccines

- Inactivated and live NDV vaccines formulated with NDV strains of low virulence, such as B1, LaSota, and more recently Ulster and VG/GA, are the most commonly administered vaccines.
- Mass application of live NDV vaccines in drinking water, sprays, or aerosols is less labor intensive than administering inactivated vaccines to individual birds, but ocular delivery provides the best response.
- Oil emulsions with inactivated antigen are the most common killed vaccine.
- Recombinant fowl pox or herpesvirus of turkeys (HVT), as live-vector vaccines to express the fusion glycoprotein of NDV, may be delivered at the hatchery in ovo or in day-old chicks.

Avian Viral Diseases

Infectious Bursal Disease

Infectious Bursal Disease

Infectious bursal disease (IBD) is an immunosuppressive disease of young chickens of worldwide prevalence.

- Etiology
- Pathobiology
- Diagnosis
- Intervention Strategies

Etiology

- IBD virus (IBDV), a double-stranded RNA birnavirus, is classified in the family Birnaviridae, genus Avibirnavirus, which is highly resistant to harsh environmental conditions.
- The virus is a single-shelled, non-enveloped virion with icosahedral symmetry and a diameter varying from 55–65 nm.
- The dsRNA of the IBDV genome has two segments designated A and B, encoding five viral proteins designated VP1, VP2, VP3, VP4, and VP5. VP2 is the protective immunogen of IBDV.

Laboratory Host Systems

Chicken Embryos

- Comparison of the allantoic sac, yolk sac, and chorioallantoic membrane (CAM) as routes of inoculation showed the allantoic sac to be the least yielding embryo-infective dose (EID50), lower than those obtained after inoculation by the CAM route. The yolk sac route gave titers that were intermediate.
- Injection of the virus into 10-day-old embryonating eggs results in embryo mortality from days 3–5 PI.

Cell cultures

- Chicken embryo fibroblasts (CEF) proved suitable to propagate egg-adapted strains of IBDV.
- Several continuous cell lines of avian origin have also been used to propagate IBDV strains including the avian fibroblastic lines DF1 and QT35.
- Normal chicken lymphocytes were the first host cells that propagated virulent IBDV.

Clinical Signs

Incubation Period

- The incubation period is short, and clinical signs of the disease are seen within 2–3 days after exposure.

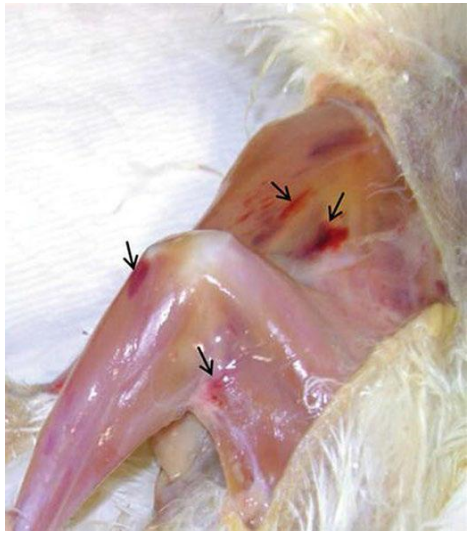
Morbidity and Mortality

- In fully susceptible flocks, the disease appears suddenly, and there is a high morbidity rate, usually approaching 100%. Mortality may be as high as 20–30%, even higher with vvIBDV.

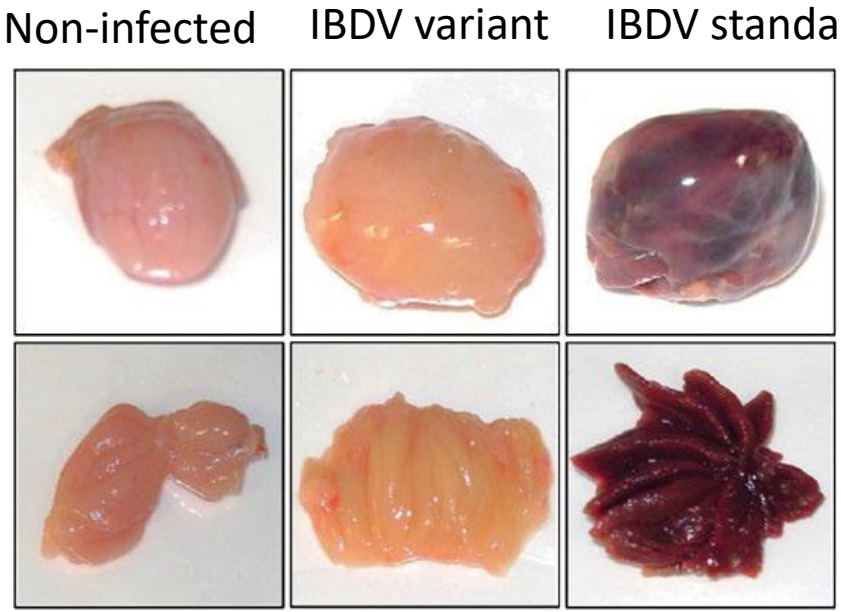
Pathology

- dehydrated, with darkened discoloration of pectoral muscles; hemorrhages are present in the thigh and pectoral muscles.
- The cloacal bursa is the primary target organ of the virus. The infected bursa often shows necrotic foci and at times petechial or ecchymotic hemorrhages on the mucosal surface.
- The spleen may be slightly enlarged and often has small gray foci uniformly dispersed on the surface. Occasionally, hemorrhages are observed in the mucosa at the juncture of the proventriculus and ventriculus (gizzard).

Gross Lesions



Hemorrhages (arrows) in thigh and breast muscles



Cloacal bursa of two-week-old SPF chickens at three days postinoculation

Diagnosis

Isolation and Identification

- The CAM of 9- to 11-day-old embryos was the most sensitive route for isolation of the virus. The embryonating egg is the most sensitive substrate for isolation of IBDV.
- Cell cultures containing 50% bursal lymphocytes and 50% CEF have been used to successfully isolate and serotype IBDV.
- Identification of the virus by direct immunofluorescent staining of affected organs or direct examination by electron microscopy has proven to be an adjunct to the isolation and identification of IBDV.
- RT-PCR was the most sensitive test for detection of the virus in bursa of experimentally infected chickens. RT-LAMP has been described as a possible alternative to RT-PCR for the molecular detection of IBDV.
- The multiplex RT-PCR or real-time RT-PCR is used for detecting and differentiating the different strains of IBDV.

Diagnosis

Serology

- The ELISA procedure is presently the most commonly used serological test for the evaluation of IBDV antibodies in poultry flocks.
- The VN test is the only serological test that will differentiate different serotypes of IBDV and it is still the method of choice to discern antigenic variations between isolates of this virus.
- The other method used for the detection of IBDV antibodies is the AGP test.

Intervention Strategies

Immunization

- Immunization of chickens is the principal method used for the prevention of IBD in chickens. Especially important is the immunization of breeder flocks so as to confer parental immunity to their progeny. Maternal antibody will normally protect chicks for 1–3 weeks.
- The major problem with active immunization of young maternally immune chicks with attenuated IBDV vaccines is determining the proper time of vaccination.
- Many choices of live vaccines are available based on virulence and antigenic diversity.
- Killed-virus vaccines are usually not practical or desirable for inducing a primary response in young chickens. Oil-adjuvant vaccines are most effective in chickens that have been “primed” with live virus.

Intervention Strategies

Immunization

- In ovo vaccination of chickens for IBD and other agents at 18 days of incubation is a labor-saving technique and may provide a way for vaccines to circumvent the effects of maternal antibody and initiate a primary immune response. The injected material is a live IBD vaccine.
- live recombinant virus vectors expressing IBDV immunogens have been reported. These include fowlpox virus, herpes virus of turkey (HVT), Marek's disease virus, chicken adenovirus, and Newcastle disease virus.

Thank you for your attention!