Avian Viral Diseases

Fuzhou Xu, PhD

Institute of Animal Husbandry and Veterinary Medicine,
Beijing Academy of Agricultural and Forestry Sciences

Avian Viral Diseases

Infectious Bronchitis

Infectious Bronchitis

Avian infectious bronchitis (IB) is an economically important, highly contagious, acute, upper-respiratory tract disease of chickens and other fowl, caused by the avian gammacoronavirus infectious bronchitis virus (IBV).

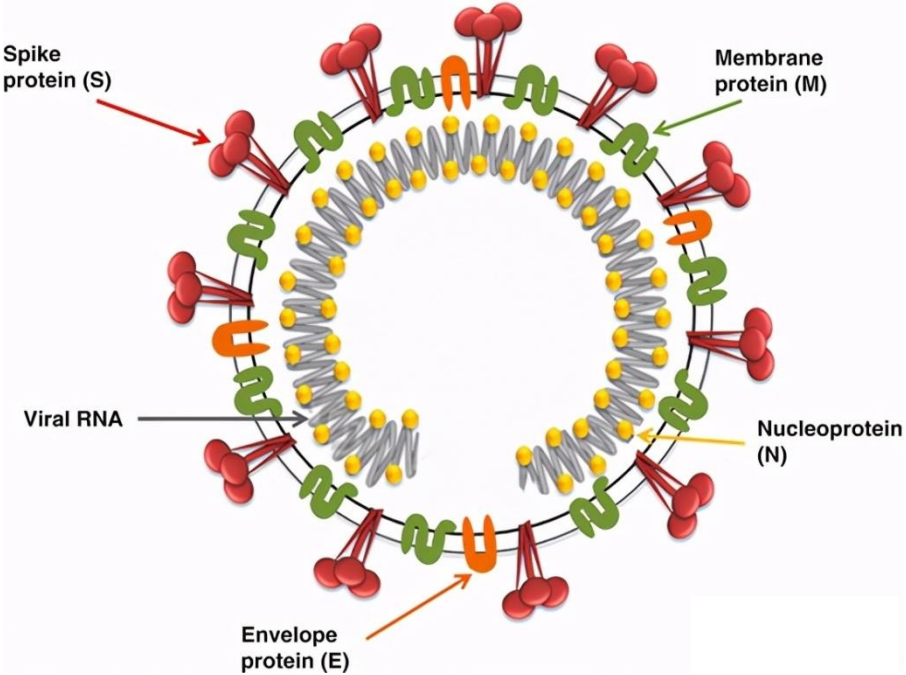
Infections, depending on the strain, may cause an acute upper-respiratory tract disease, drops in egg production, decreased egg quality, and nephritis.

Etiology

- IBV is a gammacoronavirus in the subfamily Coronavirinae and family Coronaviridae.
- IBV is an enveloped virus with a round to pleomorphic shape, with club-shaped surface projections (spikes), which gives the virus a crown-like appearance and hence the name corona.

Etiology

- The viral genome is a single-stranded positive-sense strand of RNA that is approximately 27.5–28 Kb in length. The virions are made up of spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins.



Strain Nomenclature

- **IBV strains are identified by the following scheme: IBV/bird type/country of origin/genetic type or serotype/strain designation/year of isolation.**
- **Examples:**

  China/GI-19,LX4/QX/99 (IBV and Chicken were omitted)

  IBV/Pheasant/UK/24/B171-3/99

Laboratory Host Systems

Chicken Embryos

- IBV grows well in 8-11 days of incubation in SPF chicken embryos following the inoculation of the allantoic cavity.

Cell culture

- Primary isolation of IBV field strains directly from pathological material in conventional monolayer cell cultures has proved unsuccessful.
- Chick embryo kidney (CEK) cells and chicken kidney (CK) cells show the highest sensitivity for adapted IBV strains.

Organ Cultures

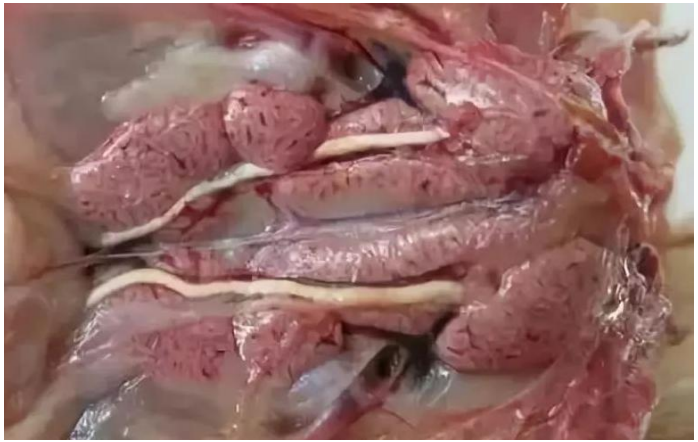
- Tracheal organ cultures (TOCs) have proved very useful for the isolation, titration, and serotyping of IBV.

Clinical Signs

- The nonspecific respiratory signs of IB in susceptible chicks are gasping, coughing, sneezing, tracheal rales, and nasal discharge.
- Broiler chickens infected with a nephropathogenic virus may show signs of depression, ruffled feathers, wet droppings, increased water intake, and mortality.
- In laying hens, IBV infections can cause egg production drops, egg quality reduction varying from loss of shell pigment, shell quality (misshapen, thin, softshelled, and rough-shelled eggs), thin to watery albumen in a fresh egg, and decreased hatchability.

Pathology

- Infected chickens have serous, catarrhal, or caseous exudate in the trachea, nasal passages, and sinuses.
- Air sacs may be foamy during the acute infection and then may become cloudy and contain a yellow caseous exudate.
- Nephropathogenic infections may produce swollen and pale kidneys with the tubules and ureters often distended with urates.



swollen kidneys with tubules and ureters distended with urates

Diagnosis

Isolation and Identification

- Tracheal swabs or fresh tracheal tissue is the preferred sample, especially within the first week of infection.
- Samples for virus isolation commonly are inoculated into the allantoic cavity of 9- to 10-day-old chicken embryos or TOCs.

Confirmation and Typing of IBV by Antibody-Based Methods

- The serotype of IBV has traditionally been determined by the VN or HI test.

Confirmation and Typing of IBV by Nucleic Acid-Based Methods

- The real-time RT-PCR test is becoming more widely used to detect IBV directly from clinical samples.
- Identification of the type of IBV in the sample is determined by sequence analysis of amplicons from the S1 gene.

Diagnosis

Serology

- The ELISA test is the most widely used serologic test for antibodies against IBV because it is inexpensive and can be used to test a large number of samples in a short time.
- Routine serology also can be done with the AGP and HI tests for detecting antibodies within the first week of infection.

Differential Diagnosis

- The clinical presentation of IB may resemble mild forms of other acute respiratory diseases such as ND, ILT, LPAI, and infectious coryza.

Intervention Strategies

Vaccination

- Because IBV is highly infectious, immunization is needed in many areas in an attempt to prevent production losses due to IB.
- Both live and inactivated virus vaccines are used for IBV immunization.

Types of Vaccine

- Live vaccines are used in broiler chickens and for the initial vaccination and priming of breeders and layer pullets. IBV strains used for live vaccines are attenuated by serial passage in chicken embryos.
- The vaccines of the Massachusetts serotype are commonly used in most countries. The goal of a vaccination program is to cover the antigenic spectrum of isolates in a particular country or region.
- Inactivated oil-emulsion vaccines are administered to breeders and layers prior to the onset of egg production.
- New “variant” strains may be used to prepare inactivated autogenous vaccines for controlling IB in laying birds.

Vaccine Delivery Methods

- **Live vaccines are usually administered individually by eye drop or intranasal application and the mass application methods including coarse spray, aerosol, or drinking water.**
- **Vaccination by spray application of maternally immune 1-day-old commercial chicks is efficacious and routinely performed, especially in the broiler industry.**
- **Application by the drinking water system, all birds can drink a sufficient amount of freshly prepared vaccine within a few hours, and should include the complete emptying of the water system before filling it with the vaccine. The water that is used with the vaccine should be of high quality, cold, and free of chemicals. The incorporation of powdered skim milk at a 1:400 concentration or another suitable product has been shown to stabilize the virus titer.**

Avian Viral Diseases

Infectious Laryngotracheitis

Infectious Laryngotracheitis

- Infectious laryngotracheitis (ILT) is an upper respiratory tract infection of chickens caused by Gallid herpesvirus type 1 (GaHV-1).
- This virus can cause severe production losses due to mortality and/or decreased egg production.

Etiology

Gallid herpesvirus type 1 (GaHV-1) is a member of the genus Iltovirus, subfamily Alphaherpesvirinae of the Herpesviridae family.

Morphology

- The GaHV-1 virion has a nucleocapsid of icosahedral symmetry surrounded by a protein tegument layer, encapsulated by an outer envelope with incorporated virus encoded glycoproteins.
- The GaHV-1 genome, contained within the nucleocapsid, is a linear double-stranded DNA molecule.

Laboratory Host Systems

Embryonating chicken eggs

- GaHV-1 was first propagated in the embryonating chicken egg.

Cell culture systems

- The virus can also be propagated in a variety of avian primary cell cultures including chicken embryo liver (CELi), chicken embryo lung (CELu), chicken embryo kidney (CEK), and chicken kidney (CK) cells.

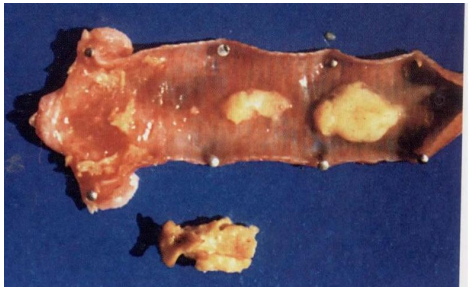
- CELi and CK cells were more susceptible to GaHV-1 infection than CEK, CELu, or embryos, since both CELi and CK cells allowed primary isolation of the virus.

Clinical Signs

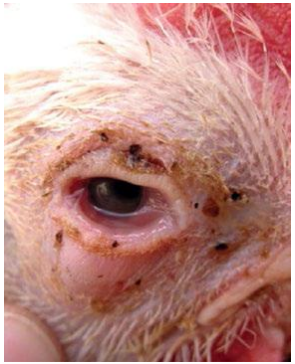
- GaHV-1 virus causes an acute respiratory disease in chickens.
- Severe forms of the disease are best characterized by increased nasal discharge, moderate to severe conjunctivitis, moist rales, followed by marked dyspnea and expectoration of blood-stained mucus.
- Virus replication causes severe epithelial damage and hemorrhages of the larynx and the trachea mucosa.
- Severe epizootics of the disease cause high morbidity (90–100%) and mortalities of 20% or higher, but usually mortalities are in the range of 5–20%.

Pathology

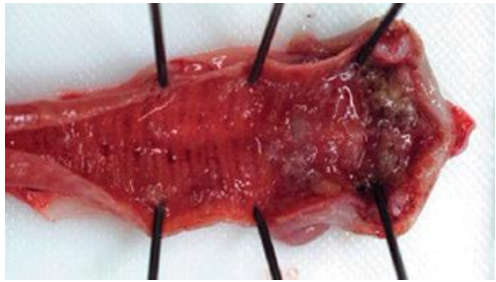
Dyspnea



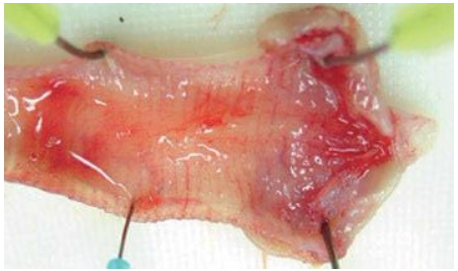
Caseous tracheal exudates



Severe and moderate conjunctivitis



Severe hemorrhagic tracheitis



Mild hemorrhagic mucous tracheitis

Diagnosis

The most common assays utilized for the rapid diagnosis of GaHV-1 infection are histopathology examination of tissues paired with real-time PCR.

Histopathology

- Lesions produced by GaHV- 1 infection are characterized by pathognomonic intranuclear inclusion bodies in respiratory and conjunctival epithelial cells. Histopathological examination is considered a rapid diagnosis.

Isolation and Identification

- Trachea, conjunctival swabs as well as larynx and lung tissues can be collected for virus isolation.
- Confirmation of GaHV-1 isolation is achieved by fluorescent antibody (FA) or immunohistochemistry (IHC) staining and PCR.

Intervention Strategies

Control of ILT generally involves routine vaccination of broiler-breeders and layers while implementing biosecurity.

Types of Vaccines

- **Live Attenuated GaHV-1 Vaccines**: Most live attenuated vaccines originated from virulent field isolates that were attenuated by sequential passage in embryonating chicken eggs and/or tissue culture.
- **Viral Vectored GaHV-1 Vaccines**: only two types of vector vaccines are currently available; the fowlpox virus (FPV) and the turkey herpesvirus (HVT).

Intervention Strategies

Vaccine Administration

- Commercial layer flocks are initially vaccinated subcutaneously at one day of age with an HVT or FPV vector vaccine followed by eye drop vaccination with chicken embryo origin (CEO) or tissue culture origin (TCO), or CEO applied in the drinking water between 8–12 weeks of age.
- Currently vaccination programs for broilers frequently consists of recombinant vaccines paired with CEO vaccines.

Avian Viral Diseases

Marek’s Disease

Marek's Disease

- Marek's disease (MD) is a common lymphoproliferative disease of chickens, usually characterized by mononuclear cellular infiltrates in peripheral nerves and various other organs and tissues including iris and skin.
- The disease is caused by a herpesvirus, is transmissible, and can be distinguished etiologically from other lymphoid neoplasms of birds.

Economic Significance

- Prior to use of vaccines, MD constituted a serious economic threat to the poultry industry causing up to 60% mortality in layer flocks and 10% condemnations in broiler flocks.
- Because vaccines are not 100% effective, sporadic losses still occur, but they are no longer as serious a problem. Vaccination for MD constitutes an outstanding example of successful disease control in veterinary medicine, and MD vaccines are the first effective vaccines against cancer in any species.

Etiology

- Marek's disease virus is an alphaherpesvirus belonging to Mardivirus genus.
- The genome consists of linear, double-stranded DNA molecules of approximately 160–180 kb.
- Several nonessential sites in MDV can be used for the insertion and expression of foreign and specific MDV genes. The anticipated advantages of MDV-vectored vaccines are that these vaccines will protect simultaneously against MD and other pathogens.

Pathotype

- Current classification schemes recognize four groups of viruses based on the virulence:
  - mild MDV -- mMDV
  - virulent MDV -- vMDV
  - very virulent MDV -- vvMDV
  - very virulent plus MDV -- vv+MDV

Laboratory Host Systems

Embryonating chicken eggs

- Virus pocks develop on the chorioallantoic membrane (CAM) of chicken embryos following yolk sac inoculation with cellular MDV preparations.

Cell culture

- Low-passage MDV grows best in duck embryo fibroblast (DEF) or chicken kidney cell (CKC) cultures, grow slowly, and produce small plaques.

Chickens

- Newly hatched chicks inoculated with virulent MDV develop gross lesions or lesions that can be detected histologically in ganglia, nerves, and certain viscera after 2–4 weeks.

Pathobiology

Incidence and Distribution

- Marek's disease exists in all poultry-producing countries.
- Sporadic outbreaks of MD occur on individual farms or regions.
- In recent years, MD has been reported as the most commonly diagnosed infectious disease among backyard chickens in multiple countries.

Natural Hosts

- All chickens are susceptible to MDV infection and tumor development. Quail, turkeys, partridges, pheasants, and some species of ducks and geese are also susceptible to infection and disease.

Pathobiology

Transmission, Carriers, Vectors

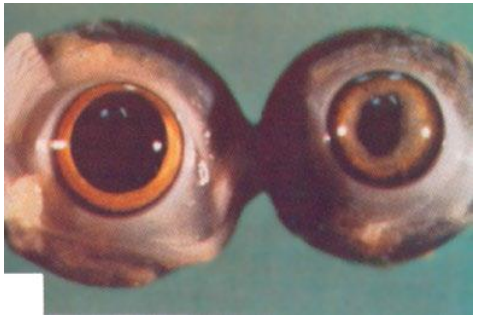
- Marek's disease virus is transmitted readily by direct or indirect contact between chickens by the airborne route.
- Feathers and dander form the major source of contamination of the environment and infection of chickens.

Incubation Period

- Under field conditions, MD outbreaks sometimes occur in unvaccinated layer chickens as young as 3–4 weeks. Most of the serious cases begin after 8–9 weeks but sometimes commence well after the onset of egg production, especially in broiler breeders or subsequent to molting.

Clinical Signs

- **Lymphoproliferative lesions, especially lymphomas, are most frequently associated with MD and have the most practical importance.**
- **skin leukosis in broilers, fowl paralysis, persistent neurological disease, and ocular lesions are additional clinical manifestations with lymphoproliferative components.**
- **A particularly characteristic clinical presentation is a bird with one leg stretched forward and the other back as a result of unilateral paresis or paralysis of the leg.**



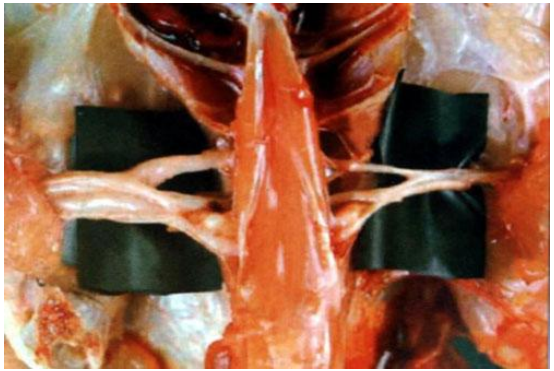
Ocular lesions on the right eye

skin leukosis

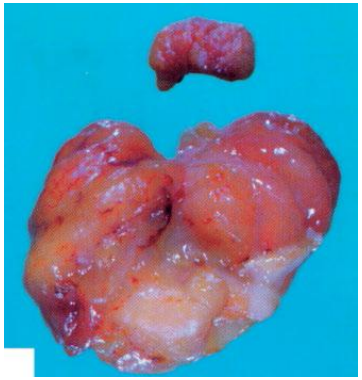
Gross Lesions

Multiple lymphomas in liver

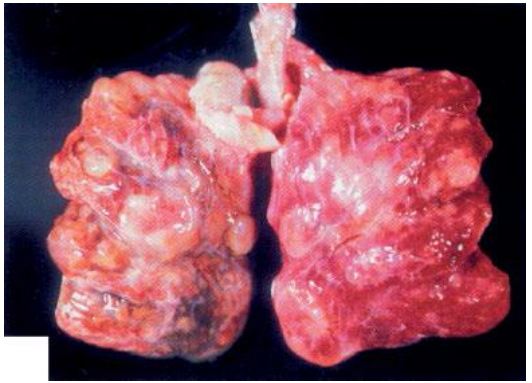
Multiple lymphomas in enlarged spleen (left)



Enlarged sciatic plexus (left) and normal plexus (right)



lymphoma in immature ovary (bottom) compared with normal ovary (top)



Multiple lymphomas in lungs

Diagnosis

Virus Isolation

- Marek's disease virus can be isolated 5 days after contact exposure and throughout the life of the chicken. Inocula may consist of blood lymphocytes, heparinized whole blood, splenocytes, or tumor cells.
- The most widely used method for primary isolation of MDV is inoculation of susceptible cell cultures with blood lymphocytes or single-cell suspensions from lymphoid tissues of infected chickens. Chicken kidney cell and DEF cultures are preferred substrates for primary isolation of MDV.

Diagnosis

Viral Antigen Detection

- Viral antigens can be detected in feather tips, cytolytically infected lymphoid tissues, brain, or infected cell cultures with appropriate antibodies by fluorescent antibody tests, immunohistochemistry, dot-ELISA, agar gel precipitin (AGP) tests, and immunoassay.

Viral Nucleic Acid Detection

- PCR-based methods for specific detection of MDV. Quantitative PCR (qPCR) assays have been used to assay viral load in tissues from infected chickens.

- DNA–DNA dot-blot hybridization with DNA probes for the detection of MDV DNA in feather tip extracts.

Diagnosis of MD

Step 1—Clinical Data and Gross Pathology

➢Chickens may be diagnosed as MD if at least one of the following conditions is met: leukotic enlargement of peripheral nerves; lymphoid tumors in various tissues (liver, heart, gonad, skin, muscle, and proventriculus) in birds under 16 weeks of age; visceral lymphoid tumors in birds 16 weeks or older that lack neoplastic involvement of the bursa of Fabricius; iris discoloration and pupil irregularity.

Step 2—Histology, Cytology, and Histochemistry of Tumor Cells

➢Histopathology can be very useful to confirm the diagnosis of lymphoma and to evaluate the morphology and distribution of tumor cells.

Step 3—Virologic Criteria

➢Viral antigen Meq can be consistently detected in tumor cells by in situ hybridization, immunohistochemistry, or fluorescent antibody tests and can be used as diagnosis criterion to confirm MD tumors.

Intervention Strategies

The development of successful vaccines for control of MD was a significant achievement. Vaccination represents, for now and the foreseeable future, the central strategy for the prevention and control of MD.

Types of Vaccines

- The most widely-used products are low pathogenic serotype 1 MDV attenuated in cell culture and naturally nononcogenic serotype 3 (HVT), and serotype 2 viruses.
- HVT, mainly strain FC126, is extensively used because it is effective and economical to produce and combines well with other products.
- Cell-associated vaccines such as the serotype 1 CVI988 strain and bivalent vaccines consisting of HVT and SB-1 or 301B/1 strains of serotype 2 MDV.
- Recombinant vaccines such as HVT vector vaccines, recombinant CVI988 vaccine with an insertion of the REV LTR.

Intervention Strategies

Vaccine Administration

- Marek's disease vaccines are administered to chicks at hatch by subcutaneous or intramuscular inoculation or in ovo at ED18.
- In ovo vaccination not only reduces labor costs and has greater precision of vaccine administration but also confers better protection against early challenge with MDV than one-day-old vaccination.
- Deposition of the vaccine by the amniotic or intraembryonic route is essential for optimal protection.
- Vaccines typically are given at doses of 2,000–6,000 plaque forming units (PFU) per chick.

Avian Viral Diseases

Adenovirus Infections

Adenovirus Infections

Avian adenoviruses fall into three genera, Aviadenovirus, Siadenovirus, and Atadenovirus.

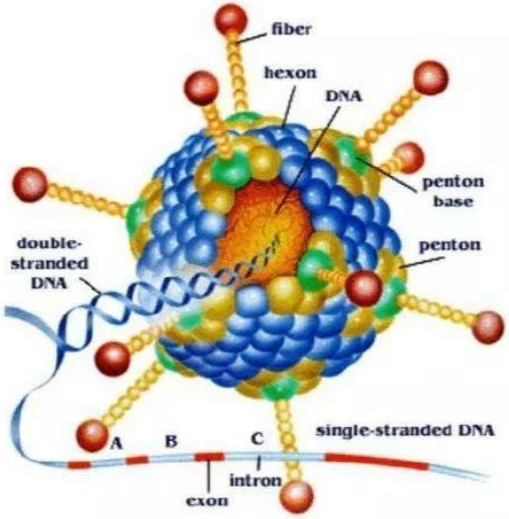
- The aviadenoviruses affecting fowl are further subdivided into 5 species (A–E) and 12 serotypes.
- Members of the Siadenovirus genus include turkey hemorrhagic enteritis virus, marble spleen disease virus.
- Atadenovirus genus includes egg drop syndrome virus.

Etiology

- Fowl aviadenoviruses (FAdV) can be separated in 5 different species (A–E) with various genotypes and 12 serotypes.

- The adenovirus virion is a non-enveloped, icosahedral structure 70–90 nm in diameter.

- The nucleic acid is double-stranded DNA, which accounts for 17.3% of the FAdV-1 virion, with the remainder being protein.



Laboratory Host Systems

Embryonating chicken eggs

- Yolk sac, in comparison to the allantoic cavity, was found most susceptible reaching the highest virus titers.

Cell culture

- Most chicken isolates have been obtained from chick kidney (CK) or chicken embryo liver (CEL) cells.
- A chicken Leghorn male hepatoma (LMH) cell line can also be used for isolation and propagation of FAdV.

Pathobiology

Incidence and Distribution

- FAdV are distributed widely throughout the world, and domestic avian species of all ages are susceptible.

Transmission, Carriers, Vectors

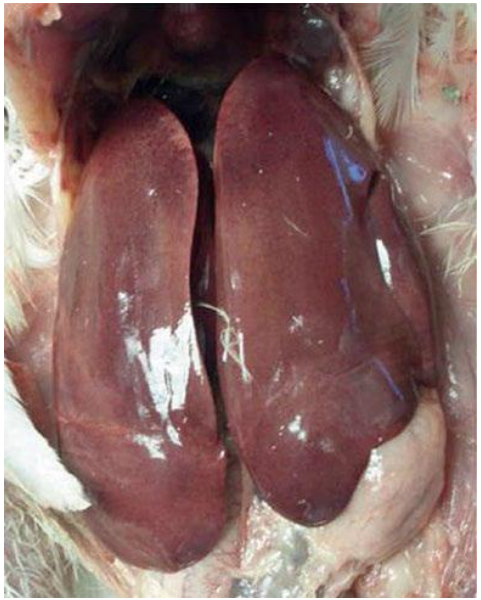
- Vertical transmission is important in the spread of FAdV and, consequently, in induction of disease.
- Horizontal transmission is another route for FAdV to spread, mainly via feces where it survives for weeks.

Clinical Signs

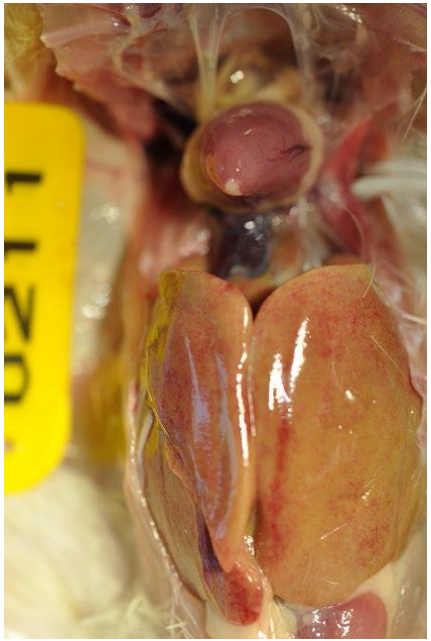
Some geno- or serotypes induce hepatitis–hydropericardium syndrome (HHS), inclusion body hepatitis (IBH), and adenoviral gizzard erosion (AGE).

- The main lesions of IBH are pale, friable, swollen livers. Small white foci can be seen on the liver. Swollen kidneys frequently coincide with glomerulonephritis.
- For HHS, gross lesions in the liver and kidneys are similar to IBH except that they are more severe.
- AGE is characterized by distended gizzards with hemorrhagic fluid and multiple black patchy erosions within the koilin layer.

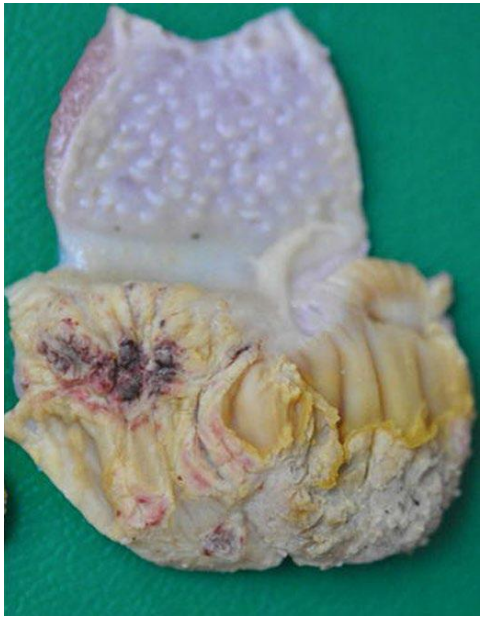
Gross Lesions



inclusion body hepatitis (IBH)



hepatitis–hydropericardium syndrome (HHS)



adenoviral gizzard erosion (AGE)

Diagnosis

Isolation and Identification

- Specimens of choice for virus isolation are feces, cecal tonsils, pharynx, kidney, and affected organs (e.g., liver, in IBH).
- A 10% suspension of tissue in medium is inoculated onto either chick embryo liver cells (CEL), chick kidney cells (CK), or on LMH cells. Embryonated eggs are insensitive for primary isolation of most aviadenoviruses.
- Immunocytochemistry, virus neutralization tests, PCR, etc. can be used to identify the virus.

Serology

- Hemagglutination is a unique feature of FAdV-1 strains. The ELISA or indirect immunofluorescence assay detect group-specific antibodies.

Intervention Strategies

Vaccination

- Autogenous vaccines are used in areas without licensed products or to improve coverage of certain strains present in the field.

- An inactivated oil-emulsion FAdV-4 vaccine was reported to induce a high level of protection against various serotypes, not only in vaccinated/challenged SPF birds but also in broilers originated from vaccinated breeders.

Avian Viral Diseases

Fowl Pox

Fowl Pox

Fowlpox is a common disease of poultry seen in many countries. Caused by the fowlpox virus, a DNA virus belonging to the Avipoxvirus genus in the family Poxviridae, the disease is characterized by production losses and cutaneous lesions.

Laboratory Host Systems

Embryonating chicken eggs

- Nine- to 12-day-old developing chicken embryos can be used for initial isolation and propagation of avianpox viruses by CAM inoculation.

Cell culture

- Avianpox viruses can be propagated in cell cultures of avian origin (e.g., chicken embryo fibroblasts, chicken embryo dermis and kidney cells, and duck embryo fibroblasts).

Birds

- A substantial degree of host specificity exists among avianpox viruses, especially those that infect wild birds.

Pathobiology

Incidence and Distribution

- Fowlpox is worldwide in distribution in commercial chickens.
- In high density areas where multiple-age birds are raised under confined conditions, the disease tends to persist for a long time despite preventive vaccinations.

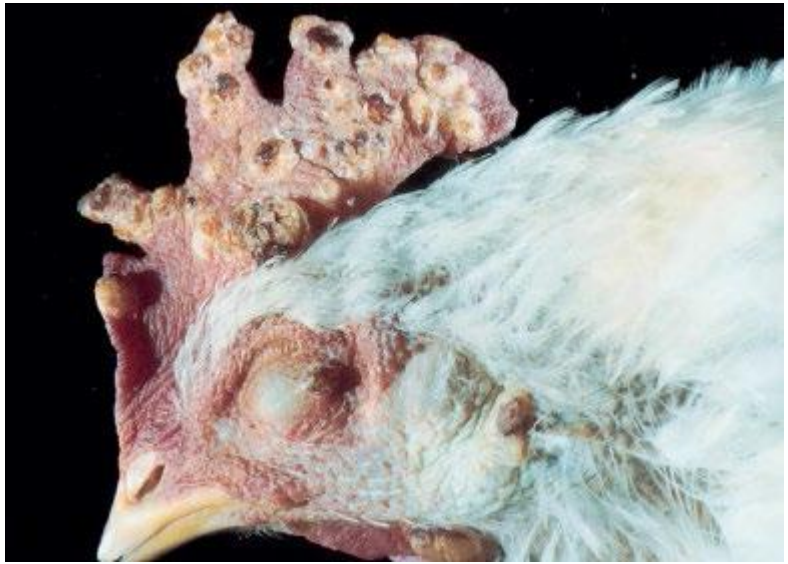
Transmission, Carriers, Vectors

- Pox virus infection occurs through mechanical transmission of the virus to the injured or lacerated skin.
- In a contaminated poultry environment, the aerosol generated by feathers and dried scabs containing pox virus particles provide suitable conditions for both cutaneous and respiratory infection.

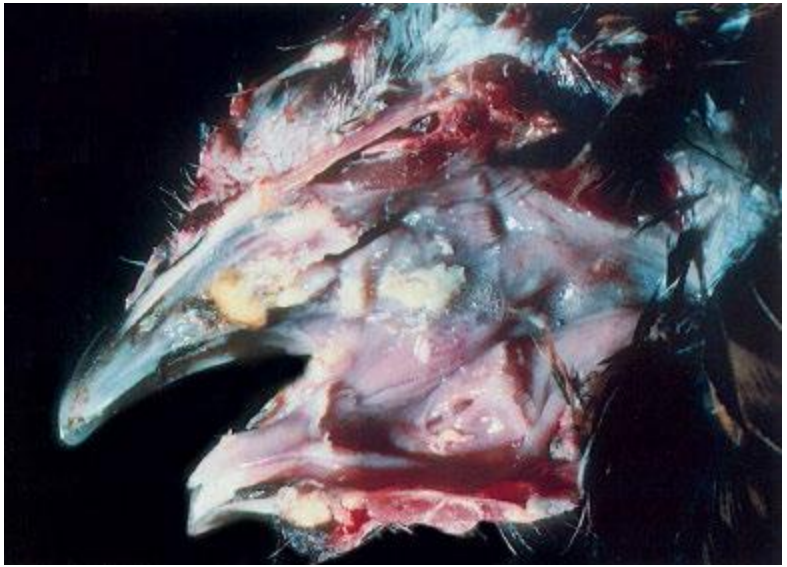
Clinical Signs

- **The disease may occur in one of the two forms, cutaneous or diphtheritic, or both.**
- The cutaneous form of the disease is characterized by the appearance of nodular lesions on the comb, wattle, eyelids, and other non-feathered areas of the body.
- In the diphtheritic form (wet pox), cankers or diphtheritic yellowish lesions occur on the mucous membranes of the mouth, esophagus, or trachea with accompanying coryza-like mild or severe respiratory signs.
- The morbidity rate of pox in chickens and turkeys varies from a few birds being infected to involvement of the entire flock. Flock mortality in chickens and turkeys is usually low, but in severe cases it may be high.

Gross Lesions



Cutaneous FWPV lesions
on the comb of a chicken



Diphtheritic FWPV lesions
in the mouth of a chicken

Diagnosis

Microscopy

- Tissue sections from cutaneous or diphtheritic lesions are processed by conventional methods followed by H&E staining for detection of cytoplasmic inclusions.

Isolation and Identification

- **Bird Inoculation**: Fowlpox virus can be transmitted readily to susceptible chickens, with typical cutaneous lesions developing in 5–7 days.
- **Avian Embryo Inoculation**: Sterile preparations of clinical samples such as cutaneous or diphtheritic lesions can be used for inoculation onto the CAM of 9- to 12-day-old developing SPF chicken embryos.
- **Cell Culture**: Primary cell cultures of chicken embryo or kidney or secondary cell cultures of avian origin can be used for virus isolation.

Intervention Strategies

Vaccination

- Vaccines of fowlpox and pigeonpox virus origin are routinely used for vaccination of chickens and turkeys in areas where the disease is endemic.
- Fowlpox vaccine is commonly applied by the wing-web method to 4-week-old chickens and to pullets about 1–2 months before egg production is expected to start.
- Attenuated FWPV vaccines of cell culture origin can be used effectively on chicks as young as one day of age.
- The flock should be examined about 7–10 days after vaccination for evidence of takes. A “take” consists of swelling of the skin or a scab at the site where the vaccine was applied and is evidence of successful vaccination.

Recombinant FWPV Vaccines

Potential of FWPV as a Polyvalent Vaccine

- Avianpox viruses have contributed significantly in the development of virology, immunology, vaccinology, and viral vector biology.

Commercially Available Recombinant FPV Vaccines

- Several live FWPV-vectored vaccines, for example, fowlpox/NDV-F-HN, fowlpox/ILTV-B, fowlpox/AIV-H5 recombinant vaccines for subcutaneous or wing-web stab immunization, are available commercially.

Avianpox Viruses as Expression Vectors for Genes from Mammalian Pathogens

- Expression of the rabies virus glycoprotein in recombinant FWPV and canarypox viruses provided a great impetus toward the use of avianpox viruses for the development of vaccines for both man and animals.

Thank you for your attention!