



## WEDNESDAY SLIDE CONFERENCE 2018-2019

### Conference 21

27 March 2019

#### Conference Moderator:

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#### CASE I: NCAH 2013-1 (JPC 4033381).

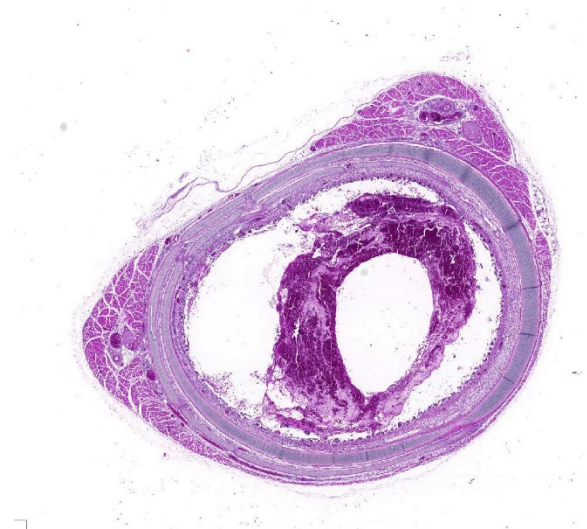
**Signalment:** 38 day old specific-pathogen-free chicken, *Gallus gallus domesticus*

**History:** The chicken was inoculated intratracheally with 0.5 mL of an experimental agent (laryngotracheitis virus). Clinical signs recorded on day 2 and 3 post inoculation include dyspnea, conjunctivitis, and depression. The bird died 4 days post inoculation.

**Gross Pathology:** On cut in, the tracheal lumen was partially obstructed by an opaque, thick blood-tinged exudate.

**Laboratory results:** N/A

**Microscopic Description:** Trachea: The lumen is 50% occluded by a coagulum composed of predominantly free erythrocytes admixed with eosinophilic proteinaceous fluid and fibrillar material (serum and fibrin), heterophils, and sloughed degenerate



*Trachea, chicken. A section of trachea is submitted the lumen contains a large clot. (HE, 18X)*

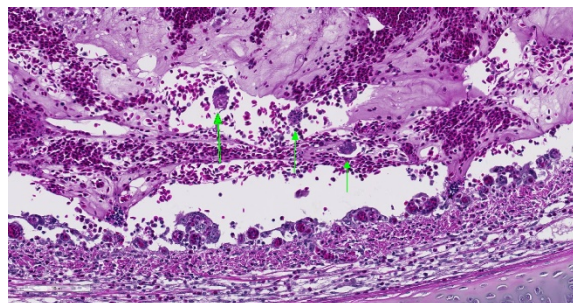
individual or syncytial epithelial cells. Syncytia contain up to 20 nuclei, often with large eosinophilic intranuclear inclusion bodies and margined chromatin. The coagulum is multifocally adhered to the extensively eroded or ulcerated tracheal mucosa with some degree of artifactual separation. Ulceration extends into the

lamina propria and submucosa with complete loss of goblet cells and projection of capillaries into the lumen. The mucosa consists of loosely adhered ciliated epithelia that often form syncytia, attenuated non-ciliated epithelium, or necrotic cellular debris. The lamina propria and submucosa are expanded by clear space (edema), fibrin, heterophils, and fewer lymphocytes and plasma cells. There is diffuse vascular congestion and multifocal hemorrhage. Within the surrounding adventitia and muscular interstitia are small numbers of predominantly lymphocytes.

**Contributor's Morphologic Diagnoses:**

Trachea: Tracheitis, hemorrhagic and ulcerative, acute, severe with epithelial syncytia and intranuclear inclusion bodies.

**Contributor's Comment:** Avian laryngotracheitis (LT) is a viral respiratory tract infection of chickens, caused by the alphaherpesvirus *Gallid herpesvirus I*, also known as laryngotracheitis virus (LTV).<sup>6</sup> The disease has a worldwide distribution, but has the highest incidence in areas of concentrated poultry production.<sup>1</sup> LT causes increased mortality and decreased egg production in chickens, leading to economic losses for the poultry industry.<sup>6</sup> The eye and respiratory tract are the usual portals of entry of LTV.<sup>6</sup> Sources of infection include chickens with acute laryngotracheitis disease, latently infected chickens shedding virus, and fomites. Clinical signs generally appear 6-12 days following natural exposure. Clinical signs in severe cases include marked dyspnea, conjunctivitis, and depression.<sup>4</sup> Severe forms of the disease cause high morbidity and variable mortality. Mild enzootic cases of laryngotracheitis cause unthriftiness, decreased egg production, watery eggs, conjunctivitis, and nasal discharge with low morbidity and mortality.<sup>6</sup>



*Trachea, chicken. The mucosal epithelium is segmentally ulcerated and attenuated, and large multinucleated viral syncytia are enmeshed within the luminal clot. (HE, 200X)*

Innate and cell-mediated immune responses appear to be more important than humoral responses in protection against disease.<sup>2</sup> Most alphaherpesviruses code for glycoprotein G which binds to a range of chemokines, modulating the host immune response, e.g. blocking binding of the chemokine to its host receptor.<sup>2</sup> In addition, glycoprotein G may enhance viral survival by modulating the host response toward humoral immunity rather than cell-mediated immunity.<sup>2</sup>

The diagnosis of LT can be made by histopathology, virus detection, or serology.<sup>1</sup> The disease is controlled through strict biosecurity measures and vaccination. Although attenuated viruses have been used by the poultry industry for years, virally vectored vaccines have recently become available, and deletion-mutant vaccines are under development.<sup>2</sup>

Differential diagnoses include infections with avian poxvirus, avian influenza virus, Newcastle disease virus, infectious bronchitis virus, fowl adenovirus, and *Aspergillus* spp.<sup>4</sup>

**Contributing Institution:**

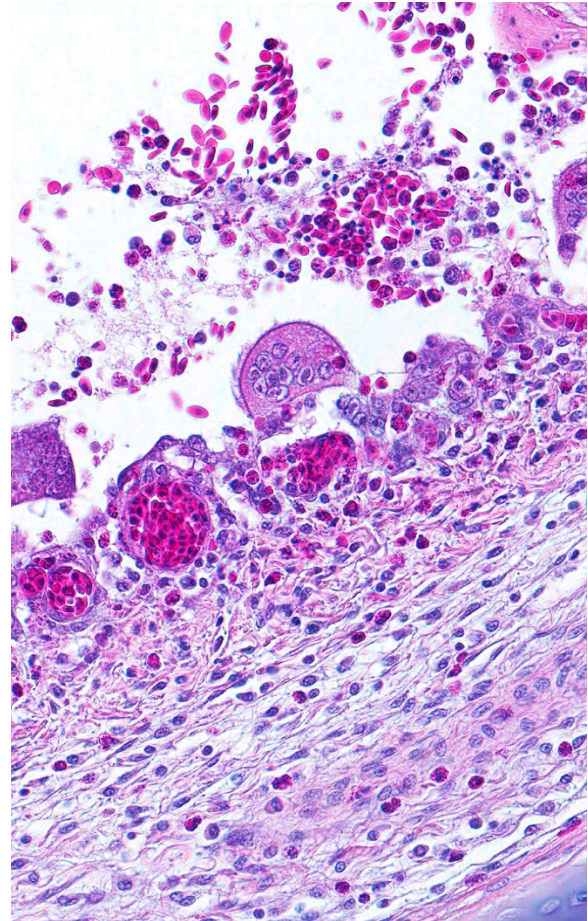
National Animal Disease Center, Ames, IA  
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[http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/about\\_nvsl.shtml](http://www.aphis.usda.gov/animal_health/lab_info_services/about_nvsl.shtml)

**JPC Diagnosis:** Trachea: Tracheitis, necrotizing and heterophilic, moderate with multifocal ulceration, epithelial intranuclear viral inclusion bodies and viral syncytia.

**JPC Comment:** The viral cause of infectious laryngotracheitis in poultry is gallid herpesvirus-1, a member of the Iltovirus (ILTovirus – get it?) genus of the subfamily Alphaerpesviridae. The disease was first reported in the 1920s, and since the advent of vaccination, two distinct clinical forms have been recognized – the classical severe form resulting in necrohemorrhagic lesions of the upper respiratory tract, and a milder form resulting in depression, reduced feed conversion and egg production, conjunctivitis, nasal discharge and sinusitis. This mild form is the most common type in the US, and is called the “vaccinal form” as analysis of the virus from many of these outbreaks demonstrate viral genome regions characteristic of vaccine strains.<sup>5</sup>

While effective in preventing infection, vaccines can also create latent carrier birds, which may spread virus to non-vaccinated flocks. As modified live vaccines may increase their virulence by bird to bird passage, especially those grown in chick embryos rather than in tissue culture, this may result in outbreaks of vaccinal forms of ILT when used over time in areas in which field-type strains are not endemic.<sup>5</sup> Moreover, CEO vaccines, often administered in drinking water, require contact with the nasal tissues during this process, and may not result in adequate coverage of birds within a given flock. This, in turn, allows for longer circulation of the virus within said flock, increased numbers of passages within birds, and ultimately predisposes to outbreaks of vaccinal laryngotracheitis.<sup>3</sup>



*Trachea, chicken. Occasionally, multinucleated viral syncytia are present within the remaining mucosal epithelium. The underlying submucosa is mildly edematous and contains moderate numbers of heterophils. (HE, 400X)*

A recent publication by Sary et al<sup>7</sup>, demonstrated plaque-like necrotic lesions in the oral cavity, buccal mucosa, and esophagus of one laying hen and one pheasant from the same mixed species flock. Mucosal necrosis, viral inclusions, and viral syncytia which demonstrated immunopositivity for ILTV were seen in the lesions. The laying hen had additional typical lesions characteristic of ILT in the respiratory tract. This was the first description of oral lesions since very early descriptions of the disease in the 1920s.

The moderator imparted his extensive experience with this disease. He mentioned that many infections are seen in older birds



up to 40 days (heavier birds which result in increased labor costs to remove from houses in outbreaks). A marked drop in water consumption occurs 24 hours prior to the break, and many birds show conjunctivitis rather than respiratory signs – for this reason, conjunctiva should always be examined as well as trachea. In the US, the disease often peaks in the winter months, but this may not be true in other parts of the world. Vaccination does not prevent infection, but may mask or hide signs of an outbreak. The moderator thinks that the presence of viral inclusions and syncytia is diagnostic and unique for this condition; infectious bronchitis is a differential in this case gross and microscopically to an extent, but will never make syncytia.

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7. Sary K, Chenier S, Gagnon CA, Shavaprasad HL, Sylvester D

Boulianne M. Esophagitis and pharyngitis associated with avian infectious laryngotracheitis in backyard chickens: two cases. *Avian Dis* 2017; 61:255-260.

#### CASE II: A-231/15 B (JPC 4066708).

**Signalment:** Meat type chicken (*Gallus gallus domesticus*, broiler), 3 weeks old.

**History:** Sudden onset of mortality affecting 10% of the flock. Sick birds adopt a crouching position with ruffled feathers and die within 48 hours.

**Gross Pathology:** At necropsy, diffuse yellowish-pale, friable and swollen livers are seen. Multiple petechiae beneath the capsule are present in some livers.

**Laboratory results:** None given.

#### **Microscopic Description:**

There is a disruption of the hepatic parenchyma due to the presence of multifocal to coalescing randomly distributed foci of degenerated hepatocytes. These hepatocytes



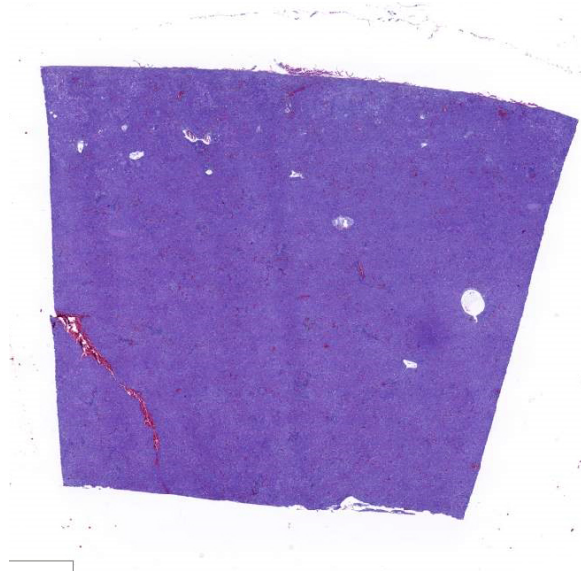
*Liver, chicken. The liver is swollen, pale, and there is a retiform pattern of necrosis. (Photo courtesy of: Servei de Diagnòstic de Patologia Veterinària, Facultat de Veterinària, Bellaterra (Barcelona), 08193 Spain.)*

are swollen and show hypereosinophilic and highly vacuolated cytoplasm, and pyknotic nucleous with karyorrhexis and/or karyolysis. Associated to these foci and randomly scattered throughout the parenchyma some hepatocytes present marked karyomegaly with chromatin condensation at the nuclear membrane and large basophilic intranuclear inclusion bodies. Moderate lymphoplasmacytic and heterophilic inflammatory infiltrate in the periportal areas is observed. Cytoplasmic vacuolation is diffusely present in remaining hepatocytes. Occasionally, focal widening and infiltration of sinusoids with lymphocytes, heterophils and histiocytes are present.

**Contributor’s Morphologic Diagnosis:**

Acute, severe, multifocal to coalescing necrotizing hepatitis with intranuclear inclusion bodies in hepatocytes

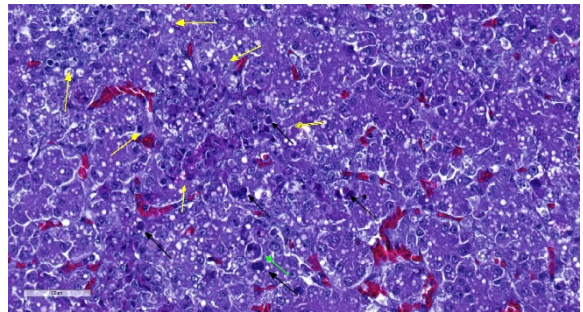
**Contributor’s Comment:** Inclusion body hepatitis (IBH) is a viral disease produced by a member of the family Adenoviridae, genus *Aviadenovirus*,<sup>1</sup> which was first described in



*Liver, chicken. A section of liver is submitted without discernible change at subgross magnification. (HE, 4X)*

chickens by Helmboldt and Frazier in 1963.<sup>3</sup> IBH is a ubiquitous disease in commercial and farm birds<sup>2</sup>, although in the last years the infection has been proven in wild and exotic birds, producing the same characteristic hepatic lesions.<sup>3</sup>

The liver is the primary organ affected.<sup>1</sup> The infection produces a multifocal necrotizing hepatitis with intranuclear inclusion bodies in the hepatocytes.<sup>1,5</sup> Intranuclear inclusion body description are variable: large, eosinophilic or basophilic, round or irregular shaped, but always replacing and displacing peripherally the chromatin, and producing karyomegaly.<sup>1</sup>



*Liver, chicken. At high magnification, there are multifocal areas of necrosis (yellow areas) infiltrated by low numbers of neutrophils, and adjacent Kupffer cells are debris-laden (green arrow). There are numerous large basophilic intranuclear inclusion within hepatocytes at the periphery of areas of necrosis as well as in the adjacent parenchyma. (HE, 400X)*

**Contributing Institution:**

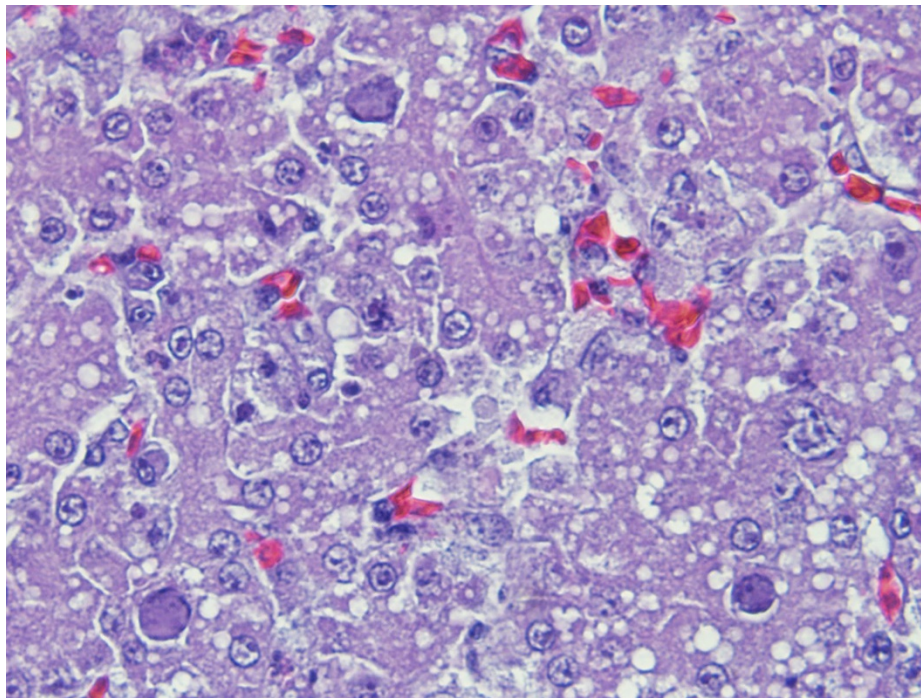
Servei de Diagnostic de Patologi Veterinaria, Facultat de Veterinaria, Bellaterra (Barcelona), 08193 Spain.

**JPC Diagnosis:** Liver: Hepatitis, necrotizing, multifocal and random, mild to moderate with numerous intrahepatocytic nuclear viral inclusions.

**JPC Comment:** Within the aviadenoviruses, there are six species (FAdV A-E and good adenoviruses, and twelve FAdV serotypes. Although all 12 serotypes

have been identified in outbreaks of inclusion body hepatitis, FAdV species D and E are most often isolated. The virus may be transmitted both vertically and horizontally, and is environmentally resistant when passed within the feces or by aerosol.

The clinical course of the disease is based on the virulence of the viral strain, age of the infected birds, as well as the presence of other viruses, to include immunosuppressive viruses such as avian circovirus and avian bunyavirus. The disease hits younger birds harder with mortalities of up to 85% in 2-day old chicks, but mortality averages 10% in 3-5 week old birds. Clinical signs are non-specific with depression and watery droppings. Grossly, livers are pale, swollen and friable. PCR for FAdV and typing is required for definitive diagnosis; the identification of FAdV in feces is not diagnostic, and FAdV are considered ubiquitous.



*Liver, chicken. Large karyomegalic inclusions are present within hepatocytes throughout the section (arrows). (HE, 400X)*

In more recent years, cases of hydropericardium syndrome, with and without associated IBH have been identified as resulting from infection with FAdV serotype 4. These cases are characterized by accumulation of straw-colored fluid within the pericardial sac, nephritis, and hepatitis with mortality rates of up to 70%.<sup>7</sup>

In addition to the inclusion body/hydropericardium syndrome, aviadenoviruses result in a number of other syndromes in chickens including necrotizing pancreatitis as well as gizzard erosions. Fowl aviadenovirus type A has been identified in outbreaks in Europe and Japan, resulting in necrosis and hemorrhage within the koilin layer, as well as adenoviral inclusions within the epithelium. Random foci of hemorrhage, unassociated with viral inclusions are also seen in this condition.

The moderator commented on the difficulties of differentiating propagating intranuclear crystalline arrays from nucleoli (which are especially prominent in turkeys), and mentioned that it is imperative to see large karyomegalic basophilic viral inclusions characteristic of adenovirus before diagnosing this condition. He also emphasized that due to the ubiquitous nature on non-pathogenic adenovirus in many flocks, isolation of an adenovirus in poultry is of little clinical importance in the



absence of disease. He also commented on the presence of glomerulonephritis in many cases of inclusion body hepatitis and the need to examine kidneys in these cases.

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#### CASE III PA 38/15 (JPC 4085106).

**Signalment:** Italian Romagnola Duck (Roman Duck), *Anser anser*

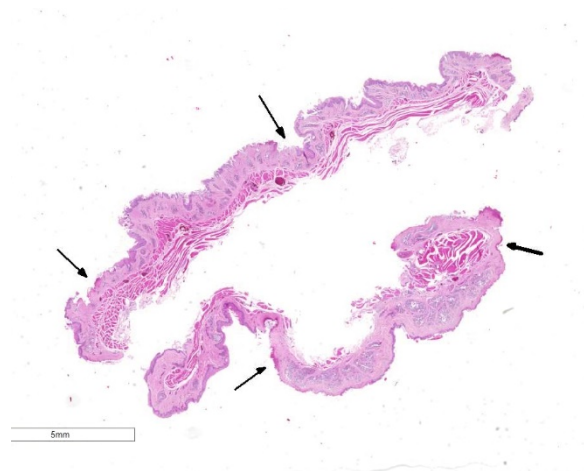
**History:** Multiple animals in the flock started dying suddenly without apparent cause or change in the management. The mortality is associated with a marked drop of egg deposition.

**Gross Pathology:** The breeder submitted only one spontaneously dead subject. The animal was a female in good postmortem and body conditions. Abundant mucous material diffusely covered the esophagus of the duck. In the proximal esophagus, beneath the mucous, multiple, multifocal to confluent, round to oval, 2 to 4mm in diameter, plaque lesions with tan-yellow discoloration and tightly attached to the mucosal surface are present. No other lesions were noted macroscopically.

**Laboratory results:** None

#### **Microscopic Description:**

Esophagus: Up to 70% (variable among sections) of the mucosal lining and 100% of the esophageal glands are severely ulcerated



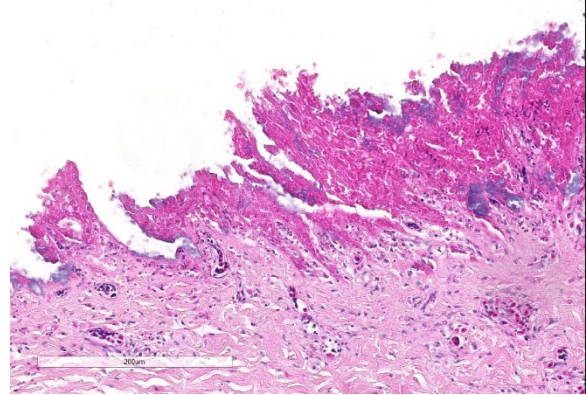
**Esophagus, duck: Multiple areas of ulceration are present within the mucosa (arrows). (HE, 4X)**

and lost with accumulation of necrotic debris, fibrin and degenerated heterophils.

In the mucosal lining, epithelial cells are variably severely sloughed and admixed with many viable and degenerated heterophils, lesser numbers of foamy reactive macrophages and lesser numbers of small mature lymphocytes in association with aggregates of 1-2  $\mu\text{m}$  basophilic coccoid bacteria. Variable percentages of residual epithelial cells have glassy homogeneous appearance with loss of cellular details and maintenance of nuclear outlines (coagulative necrosis). Occasionally, epithelial cells have 7-10  $\mu\text{m}$  in diameter, irregularly round, brightly eosinophilic intranuclear inclusion bodies that marginate the nuclear chromatin. Occasionally, intracytoplasmic inclusions are also present. Multifocally, cells of the basal layer have intracytoplasmic optically empty large vacuoles (hydropic degeneration).

Diffusely esophageal glands are ulcerated and filled by abundant pyknotic, karyolytic and karyorrhectic cellular debris (colliquative necrosis) and moderate amount of fine fibrillar material (fibrin) in association with a moderate number of foamy reactive macrophages and occasional karyolytic heterophils. In the submucosa, moderate edema, multifocal hemorrhage and mild, multifocal infiltration by a moderate numbers of heterophils and rare lymphocytes are visible. Blood vessels are diffusely and severely hyperemic.

**Contributor's Morphologic Diagnosis:** Esophagus. Severe multifocal to locally extensive acute necrotizing and ulcerative esophagitis with epithelial intranuclear and intracytoplasmic inclusion bodies consistent with herpesvirus inclusions.



*Esophagus, duck: Ulcerated areas of the mucosa are covered by a diphtheritic membrane composed of abundant granular cell debris, fibrin, small numbers of infiltrating heterophils, and numerous bacterial colonies. (HE, 80X)*

**Name of the Disease:** Duck plague or duck viral enteritis (DVE)

**Etiology:** Anatid herpesvirus-1 or DVE virus

**Contributor's Comment** The presence of esophageal necrosis, intranuclear and intracytoplasmic inclusion bodies, and the species affected were all information consistent with duck viral enteritis (anatid herpesvirus 1/AnHV-1).

Duck viral enteritis (DVE) is an acute, contagious and lethal disease that affects ducks, geese and swans.<sup>9</sup> Currently, DVE is considered one of the most widespread and devastating diseases of waterfowl of the Anatidae family because of its ample distribution, wide host range, and relatively high morbidity and mortality.<sup>13</sup> Adult ducks of Muscovy lineage (*Cairina moschata domestica*) are the most susceptible DVE.<sup>2</sup> In a recent report, the presence of DEV was found predominantly in wild ducks (*Anas platyrhynchos*) and mute swans (*Cygnus olor*), while the graylag geese (*Anser anser*), tundra bean geese (*Anser fabalis*), and grey herons (*Ardea cinerea*) were less affected.<sup>12</sup> The virus may have crossed between different orders and families and adapted to new hosts, developing the characteristic form



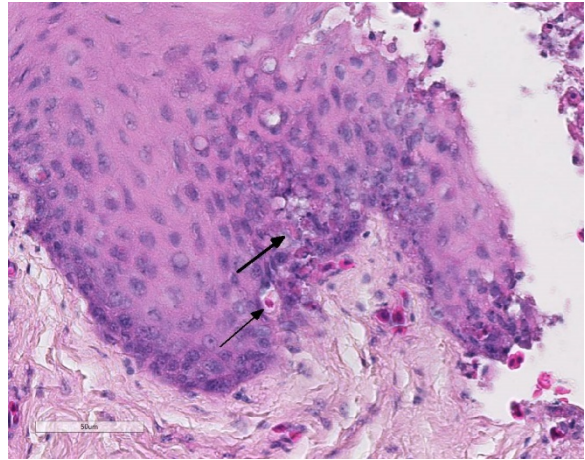
of the disease.<sup>8</sup>

Duck enteritis virus (DEV) is the causative agent of DVE and was alternatively known as anamid herpesvirus 1 (AnHV-1) and duck plague virus (DPV). Recent studies based on complete gene sequencing of Chinese virulent duck enteritis showed that the overall genome organization of DEV follows that of the alphaherpesviruses.<sup>13</sup>

DVE can be transmitted by direct contact between infected and susceptible birds or indirectly by contact with a contaminated environment.<sup>9</sup> Morbidity rate may reach 67% in flocks and mortality rate may be up to 42.2% with a drop in egg production of 45% within 4 days of disease outbreak.<sup>11</sup> Mortality usually occurs from 1-5 days after the onset of clinical signs and in chronic cases, death occurs in immunosuppressed birds. Birds recovering from disease may become carriers and may shed the virus in the feces or on the surface of eggs over a period of years.<sup>10</sup>

Clinical signs of DVE include cyanosis, lethargy, listlessness, head tilt, unwillingness to eat, photophobia, ataxia and paresis, nasal discharge, ruffled feathers, bloody diarrhea and death within 2-3 hours of onset of clinical signs.<sup>2,5,11</sup> Sudden death has been reported in several outbreaks.<sup>8,11</sup>

Gross findings include petechial hemorrhages of serosal linings, lungs, heart, liver, spleen, kidneys, pancreas, ovarian follicles, and often small and large intestinal serosa. Hemorrhagic enteritis with intraluminal bloody contents admixed with mucus, necrotizing, ulcerative and diphtheritic inflammation of the oropharynx, proximal trachea, esophagus, small intestine and cloaca accompanied by severe hepatic degeneration and multifocal necrotizing hepatitis have also been identified in several ducks.<sup>2,8,11</sup>



*Esophagus, duck: Rare mucosal epithelial cells contain intranuclear inclusions (arrow). (HE, 400X)*

Histological findings are characterized by necrosis in the digestive tract, reproductive and upper respiratory tract, liver, and spleen. Hemorrhages in digestive tract, spleen, and liver, and heterophils and macrophages infiltrating the lesions are visible.<sup>2</sup>

Esophageal lesions are characterized by swollen epithelial cells with a pale, dispersed, or vacuolated cytoplasm, individual epithelial cell necrosis or occasional larger foci of extensive epithelial necrosis and erosion and numerous large eosinophilic intracytoplasmic and intranuclear inclusion bodies.<sup>1,2</sup> Other common findings include lymphoid depletion and necrosis in the bursa of Fabricius and vascular damage in all affected organs.<sup>14</sup> Some authors describe the presence of two types of intranuclear inclusion bodies (IIBs) associated with herpesvirus infection: (a) small acidophilic IIBs surrounded by a clear halo and (b) slightly basophilic IIBs occupying the entire nucleus.<sup>8</sup>

Herpesvirus inclusion bodies are classically distinguished into two subtypes: Cowdry A inclusions are small, round eosinophilic and separated from the nuclear membrane by a halo. Cowdry B inclusions are large, glassy, eosinophilic, and centrally located, marginating nuclear material to the rim of the nucleus.<sup>6</sup>

Ultrastructurally, intranuclear non-enveloped viral particles measure about 110 nm in diameter with hexagonal shaped and variable electron-density in the cores. These are randomly distributed in the nucleus or aligned close to the nuclear envelope. The intracytoplasmic viral particles are arranged in clusters or as solitary enveloped virions measuring 200 to 250 nm in diameter.<sup>8</sup>

The definitive diagnosis of DVE is made by electron microscopy, direct immunofluorescence, PCR and immunohistochemistry.<sup>2,8,13,14</sup>

The main differential diagnoses for DVE (Anatid herpesvirus-1) include viruses responsible for both intracytoplasmic and intranuclear viral inclusions, such as cytomegaloviruses (subfamily Betaherpesvirinae), gallid herpesvirus-2 (Marek's Disease virus) and paramyxoviruses.<sup>1,3,7</sup>

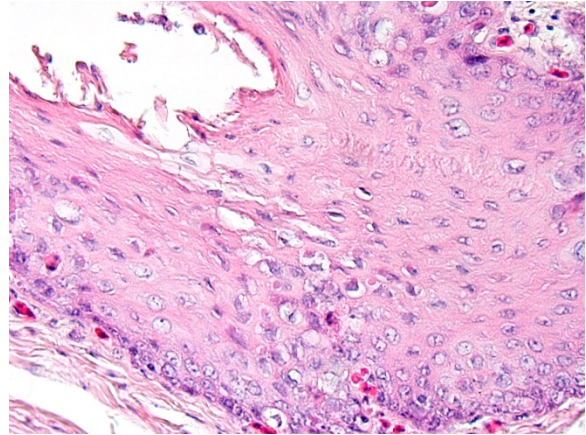
#### **Contributing Institution:**

DIMEVET-Anatomical Pathology Section  
Via Celoria 10  
20133 Milano  
ITALY

**JPC Diagnosis:** Esophagus: Esophagitis, necrotizing, multifocal to coalescing, moderate with mucosal epithelium intranuclear inclusions.

**JPC Comment:** The contributor has done an excellent job in describing this viral disease particular to waterfowl. Duck viral enteritis, first reported in the Netherlands in 1927, was originally identified as a duck-adapted strain of avian influenza until 1942, when it was identified as a distinct viral disease of ducks following fruitless transmission attempts to galliform birds, mice, guinea pigs, rabbits rats and mice.<sup>3</sup>

The virus has caused outbreaks in the US and Europe, most often in proximity to large bodies of water where wild waterfowl may



*Esophagus, duck: Rare clusters of mucosal epithelial cells contain round intracytoplasmic inclusions (HE, 400X)*

mix with domestic or farmed animals. Horizontal water-borne transmission of the virus is considered to play a primary role, with vertical transmission being of questionable importance.<sup>3</sup>

Age is a particular factor, with adult breeders showing the highest mortality and birds less than 7 days resistant to infection. Being a member of the Alphaherpesvirinae, latent infections may be seen in birds, with latent infections being maintained up to 4 years in the trigeminal ganglion, lymphoid tissues, and peripheral blood lymphocytes. Convalescent birds are immune to reinfection; however, they may maintain a latent infection and migratory birds may shed the virus, initiating new outbreaks in remote areas.<sup>3</sup>

In older birds, the characteristic lesions are those of hemorrhage due to necrosis of small capillaries and venules. In younger birds, lymphoid lesions are more prevalent. The virus induces both apoptosis and necrosis of lymphocytes in lymphoid tissues throughout the body, to include the bursa, spleen, and thymus initiating at first hemorrhagic lesions and then in convalescent birds, thymic atrophy (a sign often used in condemning

carcasses following outbreaks). Necrosis and hemorrhage of GI lymphoid tissue often results in annular bands of hemorrhage in the duodenum and ceca. The immunosuppressive effects of low-virulence duck enteritis virus (DEV) may result in an increase in secondary bacterial infections including *Riemerella anatipestifer*, *Pasteurella multocida*, and *E. coli* in younger birds. Another characteristic gross finding in outbreaks in penile phimosis.<sup>3</sup>

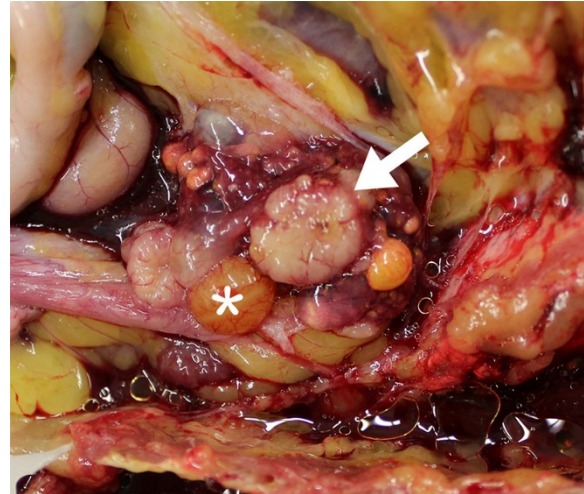
The presence of intranuclear and intracytoplasmic viral inclusions is an oddity in viral histopathology. As a DNA virus, viral replication occurs within the nucleus. The cytoplasmic inclusions are membrane-bound vacuoles containing enveloped virus and nuclei containing viral nucleocapsids.<sup>3</sup>

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*Gonad, chicken. There is a disc-shaped structure in the ventral aspect of the ovary (arrow). Developing follicles are present (asterisk). (University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources, <http://patho.uconn.edu>)*

structures, of approximately 0.9-1.2 cm in diameter x 0.3 cm deep on the ventral aspect of the ovary. The oviduct was active but underdeveloped. The left leg was swollen distal to the hock and was approximately 2-3 times the thickness of the right leg. The subcutis was expanded, wet, gelatinous and light grey to light green.

**Laboratory results:** A swab from the abnormal subcutaneous tissue from the left leg was cultured aerobically and yielded a few *Escherichia coli*, a few *Staphylococcus* sp., and a few *Streptococcus* sp.

**Microscopic Description:**

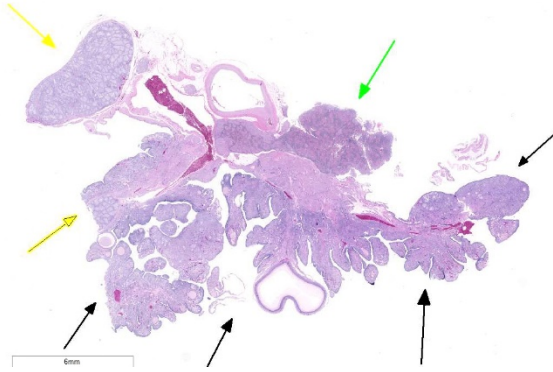
The examined gonad is comprised of both ovarian and testicular tissue, with variable degrees of differentiation. The ovarian tissue is characterized by fibrovascular stroma and finger-like projections (cortex) in which a low number of small developing follicles and one megalethical ovum are suspended. Throughout the ovarian stroma are observed multiple aggregates of lipid-laden cells (vacuolar cells), a few atretic follicles, and

**CASE IV PA 16-5448 (JPC 4101316 ).**

**Signalment:** Adult, female, Sussex chicken (*Gallus gallus domesticus*)

**History:** This adult female Sussex chicken was submitted along with two other hens; all with a reported history of lethargy, equilibrium imbalance, and watery stool.

**Gross Pathology:** At necropsy, the ovary was smaller than expected due to lack of large follicles (the largest was 1.0 cm in diameter). There were two gray/white, solid, disc-like



*Gonad, chicken. There are three distinct types of tissue in the sample – ovary (black arrows), testis (yellow arrows), and adjacent adrenal gland (green arrow) (HE, 40X).*

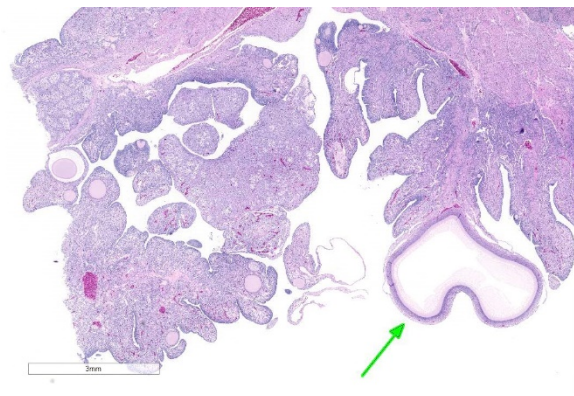
low numbers of scattered granulocytes and a few pigment-laden cells. Testicular tissue is either embedded in ovarian stroma with or without encapsulation, or attached to the ovarian tissue by a thin stalk of fibrous connective tissue. The testicular tissue is characterized by numerous, sometimes irregularly shaped tubular structures (seminiferous tubules) of varying sizes that are supported by fine to dense fibrovascular stroma. In the areas where the testicular tissue is well-demarcated/encapsulated, the tubules are fairly well-differentiated and contain developing spermatogonia, occasional multinucleated cells, and a few cells with pyknotic nuclei. Few to no late spermatids or spermatozoa are present. Adjacent to the encapsulated nodule of testicular tissue, there is a small collection of tubules lined by tall columnar epithelium (presumptive vestigial epididymis) which are devoid of spermatozoa.

Normal adrenal gland is also present along one side of the section.

**Contributor's Morphologic Diagnosis:**  
Gonad: Ovotestis

**Contributor's Comment** Disorders of sexual development (DSDs) are relatively common and affect all domestic animal

species.<sup>10</sup> The gonads, reproductive tract or external genitalia may be affected. The term intersex has been traditionally used to describe animals which exhibit some of the characteristics of both sexes.<sup>7</sup> The incidence of intersex in commercial poultry flocks has been estimated at 0.05%,<sup>1</sup> and naturally occurring sex reversal has been reported in multiple avian species.<sup>1,4,9</sup> Apparently, birds are more prone to the development of these condition for two reasons: firstly, the capability that both male and female embryos have, where cortical and medullary tissue in the developing gonads can later develop into ovary and testis respectively, is maintained after hatching.<sup>5</sup> Secondly, there is a unique asymmetry during embryogenesis in female birds,<sup>2</sup> in which greater numbers of primordial germinal cells migrate to the left gonad than to the right, that persist throughout life.<sup>4</sup> Sex reversal most commonly occurs in mature female birds that have had a pathologic condition resulting in atrophy of the ovary with subsequent development of seminiferous tubules in the normally rudimentary right gonad or in the medulla of the atrophic left ovary,<sup>5</sup> and this has been experimentally reproduced after gonadectomy.<sup>2</sup> Mammals or birds with both ovarian and testicular tissue are classified as hermaphrodites (as opposed to pseudo-

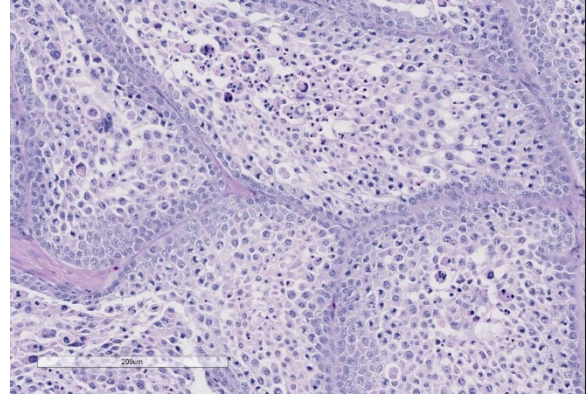


*Gonad, chicken. The ovarian tissue has a decreased number of follicles with few primordial follicles in the outer cortex, several primary follicles, and a single large yellow secondary follicle (green arrow). (HE, 80X)*

hermaphrodites which have the gonads of one sex and duct systems, external genitalia and some sexual characteristics of the opposite sex).<sup>8</sup> The ovotestis presented here is the left gonad of an adult chicken presented to us as one of three mature Sussex hens. There was evidence of follicle development and a follicular hierarchy in the ovotestis, with the larger follicles having a stigma. However, the largest follicle was about one third of the size of those of commercial layers. The oviduct was developed to about 50% of the size of a normal fully functioning oviduct. Unfortunately, it is not known if this chicken had ever laid any eggs. The testicular tissue in our case was present in the ovarian medullary stroma, sometimes well circumscribed and encapsulated, but in one area was separated from the ovary, attached only by a thin stalk of fibrovascular tissue. Interestingly, a second chicken from this batch of three, also had an ovotestis. A high incidence (2.4%) of hermaphrodites was recorded in a flock of Cochin bantams and it was suggested that there may be a hereditary predisposition for the condition.<sup>5</sup> It is not known if our two Sussex chickens were related.

In the last decade, there have been efforts to better classify DSDs by including the sex chromosome type, presence of *SRY* (in mammals; birds do not have *SRY*), gonadal type, tubular genitalia and external genital phenotype. Chromosomal typing was not performed in the present case but triploidy (ZZW) has been reported in some hens with ovotestis; triploidy can only develop at the time of fertilization.<sup>5,9</sup>

Mechanisms of sexual differentiation of the gonads appear to be highly conserved and many of the important signaling factors involved in ovarian or testicular development in mammals are also implicated in birds.<sup>2</sup> While it is known that the embryonic gonads

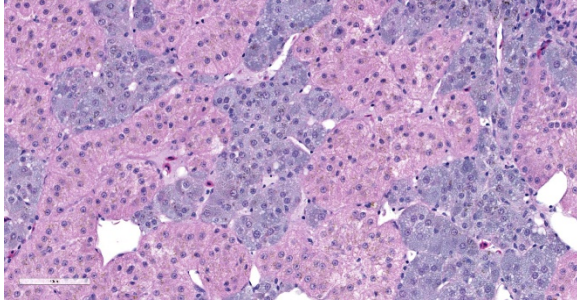


*Gonad, chicken. Testicular tissue is composed of large abnormally shaped seminiferous tissue with numerous spermatogonia but little if any mature sperm. Multinucleated cells are present within the lumina. (HE, 176X)*

in birds are bipotential for a period of time, just as they are in mammals, the sex determining system has not been fully elucidated in birds.<sup>2</sup> In mammals, the dominant acting gene is carried by the Y chromosome (*SRY* gene), and no matter how many X chromosomes are present, a single Y chromosome dictates testicular development and a genetic male gender.<sup>6</sup> In birds, the female is the heterogametic sex (ZW) while the male is homogametic (ZZ).<sup>2,3</sup> Three possible mechanisms of sex determination in birds have been proposed: A - The W sex chromosome carries a dominant female determinant, B - Dosage of one or more Z-linked genes, and C - Both mechanisms are at play.<sup>2</sup> The current weight of evidence tends to more strongly support mechanism A. For example, there are no cases of spontaneous male to female sex reversal in birds, and the loss of estrogen production by the left ovary allows the right gonad to take on a testis-like appearance and produce testosterone.<sup>2</sup>

Experimental work has also suggested that endogenous estrogens can have a determinant effect and variations in the levels of the hormone in the embryo can produce different effects. Exposure to estrogens feminizes the reproductive tract of the male embryo,





**Adrenal gland, chicken.** The adjacent adrenal gland is within normal limits. Birds do not have a discrete cortex and medulla as do mammals. (HE, 167X)

inhibition of estrogen in the female embryo disturbs development and can result in phenotypic sex reversal; both resulting in the formation of an ovotestis.<sup>2,3</sup>

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**JPC Diagnosis:** Gonad: Ovotestis.

**JPC Comment:** The contributor has done an excellent job in reviewing the normal sexual development of the chicken as well as abnormal sexual development. In chickens and all other birds, a ZZ male/ZW female sex chromosome system exists. Until day six post-fertilization, male and female gonads are morphological indistinguishable; at this point, ZZ males develop bilateral testes and in ZW females, the left gonad develops into a functional ovary. In the developing ovary, expression of the P450 cytochrome enzyme aromatase (secreted within the ovarian medulla) has been identified as the major early determinant of sexual differentiation, as its secretion catalyses estrogen secretion and subsequent ovarian development. In the

male, the enzyme SOX9, expressed within the same window, will trigger Sertoli cell differentiation and testicular development. Treatment of chicken embryos in early development with aromatase inhibitors will result in female to male sex reversal.<sup>7</sup>

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