Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

Conference 6

4 October 2017

CASE I: 3120125021 (JPC 4017935).

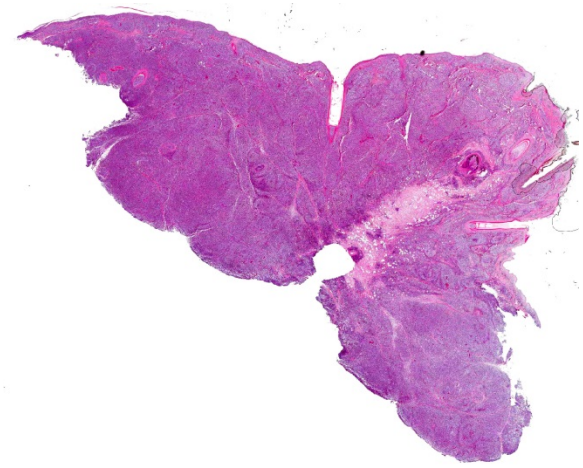
Signalment: 1-year-old, Crested Guinea fowl, *Guttera pucherani*, avian.

History: A peri-orbital mass surrounded the left eye. Growth of the mass initially started rostrally from the eye and slowly extended to the nose and the upper eyelids. Initial treatment with enrofloxacin gave transient improvement. A second treatment with the same antibiotic did not have effect. Bacterial culture yielded Staphylococci susceptible to doxycyclin. Treatment with doxycyclin was started but did not give improvement. A surgical biopsy was then taken and submitted for histopathology.

Gross Pathology: Bilaterally there was a mass of approximately 1.5cm in diameter, involving the upper eyelids and extending into the subcutis of the nose. On cut surface the mass was solid, firm and marbled pale tan to black with multifocally softer tissue with cystic spaces containing viscous mucoid material.

Laboratory results: Bacterial culture in the past: Staphylococci, not further specified.

Microscopic Description: Skin: In the dermis and subcutis, elevating the overlying epidermis, is a densely cellular, poorly demarcated, non-encapsulated, infiltrative growing, monomorphic round cell neoplasm. Neoplastic cells are organized in poorly defined lobules of large solid sheets with scant intermingled fibrovascular connective tissue. Neoplastic cells are round to oval with a small amount of pale amphophilic cytoplasm and indistinct cell borders. Nuclei are round and contain coarsely clumped chromatin and one or two



Feathered skin, guinea fowl. At subgross magnification, the dermis is expanded and largely effaced by sheets of neoplastic round cells. (HE, 5X)

moderately distinct nucleoli. Mitotic figures are 18 per 5 HPF and there is mild anisocytosis and anisokaryosis. Small numbers of neoplastic cells multifocally infiltrate the epidermis and feather follicle epithelium and there's multifocal infiltration and replacement of pre-existing muscle tissue and lacrimal glands (not present in all slides). Multifocally there are areas of necrosis characterized by pyknosis, karyorrhexis, karyolysis, loss of cellular detail and amorphous, eosinophilic debris with associated infiltration of moderate numbers of heterophils. Multifocally dispersed throughout the neoplasm there are small numbers of heterophils and macrophages with an occasional multinucleated giant cell. The overlying epidermis is multifocally ulcerated and covered with serocellular crusts (not present in all slides).

Contributor's Morphologic Diagnosis:
Skin: Lymphoma.

Contributor's Comment: Guinea fowl are terrestrial, ground-living birds that belong to the order Galliformes, family Numidae. Some ornithologists consider them to be part of the superfamily Phasanidae (including Partridges and Pheasants).

There are limited reports available on lymphoid neoplasms and neoplasms in general in guinea fowl. Pancreatic adenocarcinomas and seminomas have been reported as rare spontaneous neoplasms in guinea fowl.[3,1] Viral- induced pancreatic tumors and duodenal adenomas have been reported in guinea fowl in experimental settings.[9,2] In general, the most common naturally-occurring neoplasms in birds are lymphomas, fibromas and fibrosarcomas, and lipomas[6]; however these information are based upon a population including species of the Galliformes order but not including domestic fowl.

Lymphomatous tumors are also relatively common in domestic fowl and turkeys. Common sites of occurrence are liver, spleen, thymus and kidneys. Cutaneous and oropharyngeal lymphomas have been reported in pheasants. The neoplasms were present in the skin around the eyes, around the external ear openings and involving the hard palate.[4] Histologically, these tumors were composed of a pleomorphic mixture of lymphoblasts and lymphocytes. [4]

A similar case of a guinea fowl presenting with a periorbital lymphoma has been reported and was associated with visceral lymphomas in the spleen, lungs, kidneys, liver and ovary. [8] In our case, however, we did not perform a full necropsy and information regarding the internal organs was not available.

Most information on ocular and periorbital tumors in animals is from dogs and cats. In these animals, ocular tumors are relatively rare. Ocular tumors can arise from the eyelids and adnexa, the optic nerve and structures within the globe, and metastases are generally infrequent. The most frequent neoplasms of the eyelid and conjunctiva in dogs are squamous cell carcinoma and Meibomian adenoma. Less frequently encountered are melanocytic neoplasms, papillomas, vascular tumors, mast cell tumors and lymphomas.

In dogs, cats and cattle, multicentric lymphoma regularly involves the eye. In dogs and cats, there is predominant involvement of the uvea, whereas in cattle retrobulbar (orbital) neoplasms are more common. Only occasionally are lymphomas encountered in the tissues of the eyelids in these animals. Solitary lymphomas in the

conjunctiva and third eyelid have been reported in cats and horses.

In birds, squamous papillomas, squamous cell carcinomas, malignant melanomas, basal cell tumors and adeno(carcino)mas of the lacrimal gland have been reported in the skin and subcutis of the eyelids.[7] Lymphoma is reported as a neoplasm arising in the orbit as are infiltrative carcinomas, chondromas and teratomas.[7] An infectious etiology has not been identified in the lymphomas reported in pet birds or in the report of a periorbital lymphoma in a Guinea fowl.[7,8]

Lymphocytic neoplasms in poultry are often categorized as infectious or non-infectious. Spontaneously occurring neoplasms often involve older birds whereas viral induced neoplasms develop in relatively young birds.[5] The most important viral-induced neoplastic diseases in poultry are:

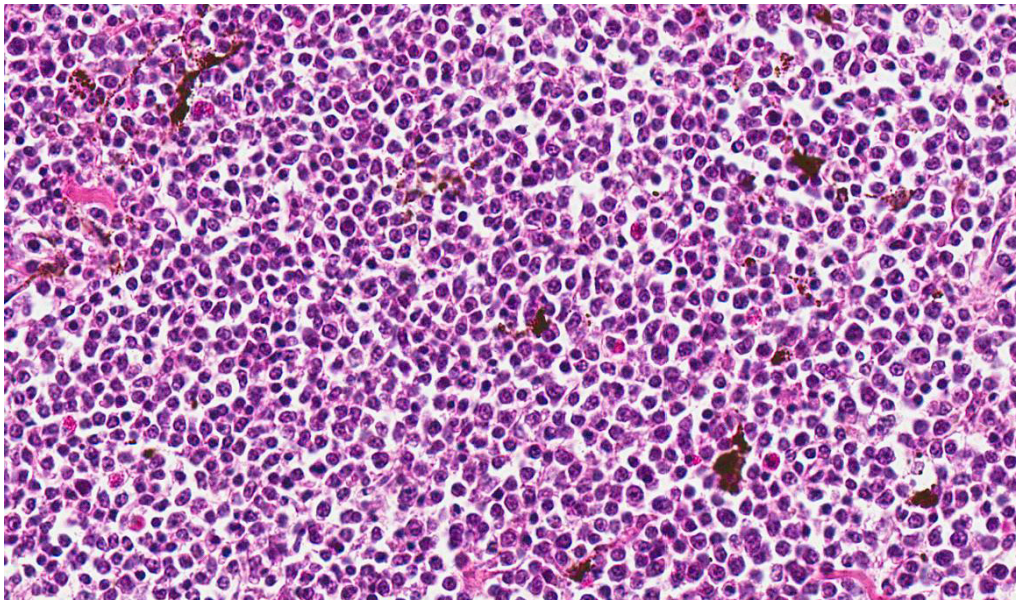
- Marek's disease, caused by Gallid herpesvirus type 2
- Avian Leukosis caused by avian leukosis virus (retrovirus)
- Reticuloendotheliosis, caused by reticuloendotheliosis virus (retrovirus).

Of these entities, Marek's disease can involve the skin.[5] Marek's disease is a disease that mainly affects chickens and only occasionally affects pheasants, quail, game fowl and turkeys. To the best knowledge of the author, Marek's disease has not been reported in guinea fowl.

JPC Diagnosis: Feathered skin: Lymphoma, crested guinea fowl (*Guttera pucherani*), avian.

Conference Comment: There are three main viral neoplastic diseases in chickens which result in lymphoid tumors: Marek's disease (MD), avian leukosis, and reticuloendotheliosis.[5]

Marek's disease is caused by an alpha herpesvirus (Gallid herpesvirus-2) and typically affects young chickens, and rarely quail, turkeys, pheasants, and jungle fowl. The herpesvirus that causes MD is classified into three serotypes. Serotype 1 is ubiquitous in chickens and varies in pathogenicity from very virulent (vv+) which is oncogenic to avirulent (mild). Serotype 2 is also common in chickens but is



Feathered skin, guinea fowl. Neoplastic cells have distinct cell borders, a moderate amounts of finely granular eosinophilic cytoplasm, irregularly round nuclei with coarsely stippled chromatin. Moderate numbers of heterophils and plasma cells are scattered throughout the neoplasm. (HE, 400X)

non-oncogenic and serotype 3 is common in turkeys and also non-oncogenic. The virus is spread through inhalation of virus-containing feather follicle dander of infected birds which can spread across long distances. Carrier birds can be silently infected and periodically shed the virus throughout their lives. There are four different types of lesions seen in MD: (1) peripheral nerve enlargement, (2) discoloration of the iris; (3) swelling of feather follicles with skin reddening (leukosis); (4) and visceral tumors often involving heart, spleen, liver, gonads, kidneys, and proventriculus. Of the four, visceral tumors are most common and can result in depressed, cachexic birds prior to death with vague clinical signs. Microscopically, lymphomas caused by MD contain pleomorphic T-lymphocytes that carry a MD tumor-associated surface antigen (MATSA).[5] In pet birds, epitheliotropic lymphoma that appears similar to MD occurs but the etiology has not been identified.[11]

Avian leukosis (ALV) affects mature chickens and is caused by alpha retroviruses known as avian leukosis viruses which have been further classified into 10 subgroups (A through J). Retroviral strains are classified by the pathogenicity of their lesions and subgroup. Lymphoid leukosis (LL) is the most common, caused by ALV subgroup A, and characterized by gradual onset, low mortality, and neoplasia of the Bursa of Fabricius with metastasis to other visceral organs. Recently a new strain of ALV was discovered, "J", which causes myeloid leukosis (myelocytomatosis) and most likely results from recombination of endogenous and exogenous retroviruses. In contrast to MD, egg transmission is the predominate mechanism of spread in ALV. These chicks are immune tolerant and do not develop antibody but have an increased risk of death from LL. If female, these chickens will lay fewer eggs and shed the virus to their own progeny further disseminating the infection. Clinical signs of ALV are non-specific and many birds simply appear emaciated and lethargic. Tumors may be detected within the bursa of Fabricius by insertion of the clinician's finger into the cloaca. Additionally, birds with skeletal myelomatosis (subgroup J) may develop osteopetrosis of the shanks resulting in what are colloquially known as "boot shanks". Avian retroviruses cause osteopetrosis by infecting osteoblasts and making them constitutively active, while osteoclasts remain unaffected.[1,2] Microscopically, neoplastic cells are uniformly lymphoblastic and positive for immunoglobulin M and B-cell markers since they originate in the Bursa of Fabricius.[5]

Reticuloendotheliosis (RE) encompasses a variety of conditions caused by retroviruses. Only two conditions caused by this non-defective RE virus, runting syndrome and chronic lymphoma are of economic importance particularly in the southern U.S. Runting disease is induced by vaccination with RE virus-contaminated biologics of chicks less than 1 week old and characterized by stunted growth, feather abnormalities, and severe atrophy of the thymus and Bursa of Fabricius leading to immunosuppression and concurrent infections. Chronic lymphoma syndrome occurs very rarely in turkeys, ducks, quail, pheasants, geese, peafowl, prairie chickens and chickens and results in few clinical signs other than mild depression prior to death. In experimentally infected animals lesions resemble LL or MD.[5]

Diagnosis of lymphoid tumors in birds can be difficult and necessitates a complete history, thorough necropsy, immuno-

fluorescent tests for surface antigens, and molecular techniques like PCR.[5]

Contributing Institution:
http://www.uu.nl/faculty/veterinarymedicine/EN/labs_services/vpdc

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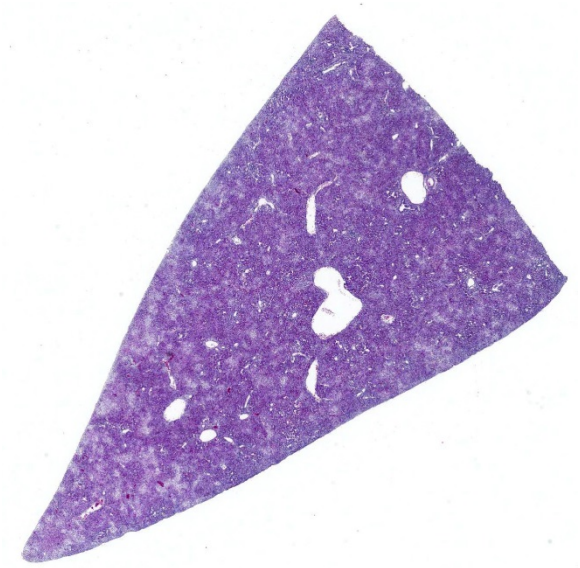
CASE II: 12060787 (JPC 4033563).

Signalment: 26-day-old, male and female, commercial white broiler chickens, *Gallus gallus domesticus*, avian.

History: Five birds were submitted from a commercial broiler facility for evaluation for gangrenous dermatitis. Three birds were deceased and two were alive at time of submission. No other history available.

Gross Pathology: Per submittal request, there were no lesions consistent with gangrenous dermatitis in the birds. Instead, the livers were swollen, friable, pale and riddled with dark red to light yellow military foci. Additionally, the kidneys were pale, swollen and friable and the spleen was mottled.

Laboratory results: Acid fast stains on tissues were negative.



Liver, chicken. At subgross magnification, a retiform pattern of pallor (necrosis) is present throughout the section. (HE, 5X)

Microscopic Description: Liver: The hepatocellular parenchyma is interrupted by numerous, individual and coalescing foci of necrosis. The foci of necrosis are abruptly demarcated from surrounding viable parenchyma and contain necrotic cell debris along with infiltrates of intact and degenerate heterophils and scant erythrocytes (hemorrhage). Typically restricted to the margin of the viable: necrotic parenchyma, several hepatocytes exhibit large, granular to homogeneous, basophilic intranuclear inclusion bodies that peripherally displace chromatin. Lastly, multifocal portal regions have mild to moderate infiltrates by lymphocytes, plasma cells and fewer macrophages.

Contributor's Morphologic Diagnosis:
Liver: Marked, acute to subacute, necrotizing hepatitis with basophilic, intranuclear, inclusion bodies consistent with adenovirus (inclusion body hepatitis).

Contributor's Comment: Inclusion body hepatitis (IBH) was first reported from an outbreak in chickens in the United States by Helmboldt and Frazier.[3] Since that time, IBH has grown to become a ubiquitous disease with worldwide distribution. IBH occurs in chickens 3-7 weeks of age and is characterized by an abrupt increase in mortality that lasts 3-5 days and can approach 10-30%.[2,5] Affected birds exhibit an enlarged mottled liver, icterus, hemorrhages, and pale, swollen kidneys. Microscopic evaluation shows hepato-cellular necrosis with eosinophilic or basophilic intranuclear inclusion bodies.

Inclusion body hepatitis is caused by fowl adenovirus. Fowl adenoviruses are divided into at least 12 serotypes, most of which are apathogenic. IBH is typically associated with serotypes 2 and 8; whereas a different clinicopathological manifestation of fowl adenovirus infection, known as hydro-pericardium syndrome (HPS), is typically associated with serotype 4.[2,5] However, some birds have demonstrated both IBH and HPS from administration of the same strain[4],and therefore, the clinical disease form may be multifactorial related to background, co-infections, route of infection or age of the bird.

Historically, outbreaks of IBH have been associated with concurrent disease, particularly immunosuppressive agents such as infectious bursal disease (IBD) or chicken anemia virus (CAV).[1,6] In the present case, cloacal bursas from affected birds were examined at necropsy and microscopically; no lesions were identified. Additionally, serologic surveys on affected flocks did not demonstrate IBD infection in birds dying from IBH.

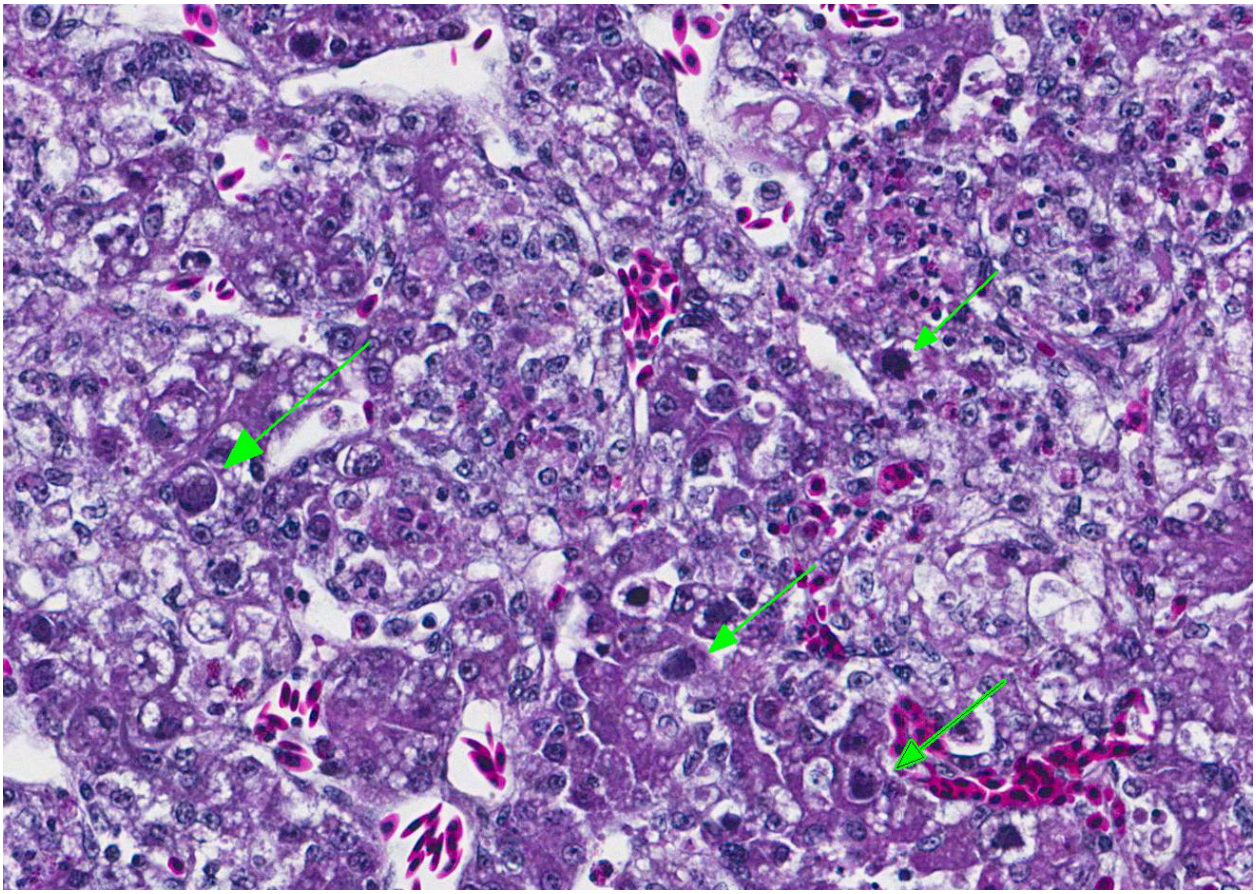
A PubMed search for information related to IBH returns a paucity of contemporary studies. Therefore, although this disease may no longer be commonly seen or studied, similar to infectious canine hepatitis (canine

adenovirus-1), IBH does re-emerge on occasion.

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, marked with numerous basophilic intranuclear viral inclusions, *Gallus gallus domesticus*, avian.

Conference Comment: There are three genera that affect birds: (1) Aviadenoviruses (group I) which contain fowl adenoviruses (IBH), goose adenoviruses, falcon adenovirus 1, duck adenovirus 2, pigeon adenovirus 1 and turkey adenovirus 1 and 2; (2) Siadenoviruses (group II) which contain turkey adenovirus 3 (hemorrhagic enteritis, marble spleen disease) and raptor adenovirus 1; and (3) Atadenoviruses (group III) which contains duck adenovirus 1 (egg drop syndrome).[6] These ubiquitous viruses generally only cause disease in immunosuppressed birds.

Fowl adenovirus, the cause of inclusion body hepatitis, has worldwide distribution, typically affecting young chickens. Most animal species have their own adenovirus that induces hepatitis, enteritis, or respiratory disease (see chart below). With IBH, gross lesions are usually non-specific and may consist of pallor of the wattles and comb, depression, and a sudden increase in mortality within the flock. Depending on the severity of the hepatitis, there may be petechial and ecchymotic hemorrhages in the skeletal muscles of the legs. The liver is generally enlarged with mottling characterized by soft, yellow, focal areas and hemorrhage. Kidneys are often swollen



Liver, chicken. Adjacent to areas of hepatocellular necrosis and loss, the nuclei of degenerating hepatocytes are expanded by a single basophilic viral inclusion (arrows). (HE, 400X)

and mottled as well and the Bursa of Fabricius is usually reduced in size. Microscopically, there is focally extensive degeneration and necrosis of hepatocytes with the characteristic basophilic intra-nuclear viral inclusion bodies within hepatocytes adjacent to areas of necrosis. Renal lesions consist of membrano-proliferative glomerulonephritis, and the Bursa of Fabricius is grossly small due to lymphoid depletion.[6]

Hemorrhagic enteritis of turkeys (caused by turkey adenovirus) results in large basophilic intranuclear viral inclusion bodies within cells of the mononuclear phagocyte system in the spleen leading to widespread necrosis and involution of the white pulp. There is lymphoid depletion in the thymus and Bursa of Fabricius. Intestinal lesions are most prominent in the duodenum and are characterized by mucosal congestion, degeneration and necrosis of the epithelium lining the villus tips and luminal hemorrhage with mixed inflammation in the lamina propria. Adenoviral inclusions are rarely seen in intestinal epithelia, liver, bone marrow, circulating leukocytes, lung, pancreas, brain, and kidney.[6]

Egg drop syndrome is caused by a hemagglutinating adenovirus (duck adenovirus 1) and results in loss of color of pigmented eggs, decrease in production, or production of thin-shelled, wrinkly eggs in healthy looking laying hens. The virus is widespread in its natural host, waterfowl, and spread vertically and horizontally to domestic birds. In chicks infected in utero the virus remains latent until they start laying eggs. The primary site of replication is in the pouch shell gland and gross lesions other than atrophied ovaries and oviducts are not appreciated. For diagnosis, allantoic fluid can be checked for hemagglutinating activity or viral DNA detected by PCR.[6]

Table 1: Select adenoviruses in veterinary species[4]

Species	**Name/Species**	**Comment**
Dogs	Canine adenovirus 1 Canine adenovirus 2 (mastadenovirus)	- Infectious canine hepatitis - Infectious canine tracheobronchitis
Horses	Equine adenovirus 1 & 2 (mastadenovirus)	- Asymptomatic or mild respiratory disease in immunocompetent hosts - Bronchopneumonia/systemic disease in Arabian foals with SCID
Cattle	Bovine adenovirus (mastadenovirus and atadenovirus)	- 10 serotypes - Asymptomatic or mild respiratory disease - Occasionally pneumonia, enteritis, keratoconjunctivitis in calves
Swine	Porcine adenovirus (mastadenovirus)	- 4 serotypes - Asymptomatic or mild respiratory disease/enteritis; rarely encephalitis
Sheep	Ovine adenovirus (mastadenovirus and atadenovirus)	- 7 serotypes - Asymptomatic or mild respiratory disease - Occasionally severe respiratory/enteric disease in lambs

Goats	Caprine adenovirus (mastadenovirus and atadenovirus)	- 2 serotypes - Asymptomatic or mild respiratory disease
Deer	Cervine adenovirus (Odocoileus adenovirus 1; atadenovirus)	- Vasculitis, hemorrhage, pulmonary edema
Rabbits	Adenovirus 1 (mastadenovirus)	- Diarrhea
Mice	Murine adenovirus 1 & 2 (mastadenovirus)	- Murine adenovirus 1: experimental infections - Murine adenovirus 2: enterotropic; causes runting in neonates
Guinea pigs	Guinea pig adenovirus (mastadenovirus)	- Usually asymptomatic; rarely pneumonia with high mortality, low morbidity

Conference attendees also discussed two viral agents that cause immunosuppression and are common concomitant infections in IBH cases: chicken infectious anemia and infectious bursal disease (IBD).

Chicken infectious anemia (*Gyrovirus* genus, Circoviridae family) is a disease of young birds that is characterized by aplastic anemia, generalized lymphoid atrophy, intramuscular hemorrhage, and immuno-suppression. Older birds are usually not affected unless the animal has concurrent infection with infectious bursal disease. The most common method of transmission is vertical from infected hens. Also transmission in feces is common with crowded bird houses. The most common gross lesions are thymic atrophy and yellow, fatty bone marrow. Microscopically, there is thymic lymphoid depletion and atrophy of all cell lines within the bone marrow. Due to resulting immunosuppression, secondary bacterial infections like gangrenous dermatitis may be present.[6]

Infectious bursal disease (also known as "Gumboro disease") is caused by avian birnavirus and results in inflammation and atrophy of the Bursa of Fabricius with resulting immunosuppression. Avian birnavirus has two serotypes with serotype 1 being pathogenic. The virus spreads rapidly, is persistent in the environment, and infects chickens as long as they have a functional Bursa of Fabricius (1-16 weeks old). Initial clinical signs are vague: dehydration, diarrhea, tremor, ataxia, depression, anorexia, and a droopy appearance are common and may resemble coccidiosis. Microscopically, there is marked lymphoid necrosis within the Bursa of Fabricius, thymus, Harderian gland, cecal tonsils, and Peyer's patches followed by atrophy and replacement with fibrous connective tissue.[6]

It was originally thought that IBH was always be preceded by an immuno-suppressive pathogen but recently IBH has been accepted as a primary disease.[6]

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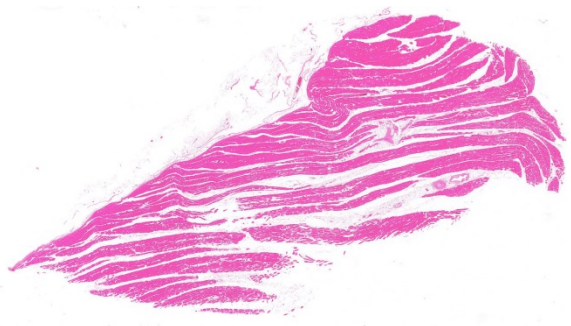
CASE III: T (JPC 4035426).

Signalment: 12-month-old, female, breeder hen turkeys, *Meleagris gallopavo*, avian.

History: A spike in mortality was reported in this flock of 2200 breeder hens. Affected live birds were down, unable to rise, had drooped wings and were panting. In the tom barn, the birds were using their wings to stand. Both live and dead birds were submitted to the local field veterinarian for postmortem examination.

Gross Pathology: The veterinarian noted that the majority of the birds had pale streaking of the breast and thigh muscles and some birds also had pale streaking of the myocardium. Formalin-fixed tissues, frozen tissues, bacteriology swabs and a feed sample were submitted to the laboratory for further testing**.**

Laboratory results: PCR testing of lung/trachea was negative for Avian Influenza and Avian Paramxyovirus-1 infection.
Bacterial culture of swabs from bone marrow and peritoneum was negative for bacterial pathogens.
Feed sample HPLC Ionophore Analysis: 36 ug/g salinomycin was detected. Levels of



Skeletal muscle, turkey. A single section of skeletal muscle is presented for examination. There are small areas of pallor within the muscle scattered randomly throughout the section (HE, 6X)

monensin, narasin and lasalocid were < 1 ug/g.

Microscopic Description: Skeletal muscle: Sections from the skeletal muscle from the thigh reveal widespread acute myonecrosis, with swelling, hyalinization, fragmentation and hypercontraction of myofibres. Numerous coagulated portions of myofibres are lightly mineralized and pale eosinophilic lightly fibrillar material is often present between the retracted necrotic myofibres. There is very early hypertrophy of satellite cell nuclei and low numbers of infiltrating macrophages and heterophils.

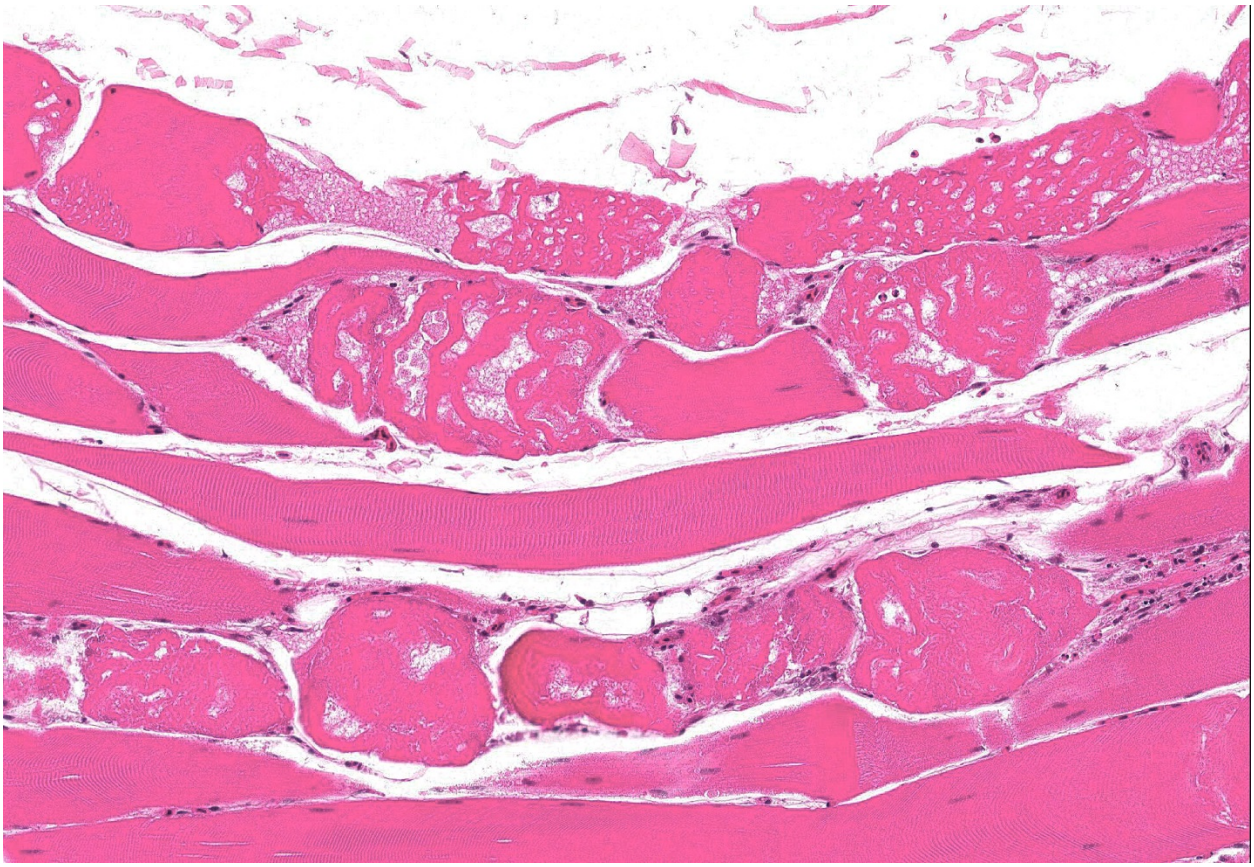
Contributor's Morphologic Diagnosis:
Skeletal muscle: Acute severe multifocal monophasic skeletal myonecrosis.

Contributor's Comment: Differential diagnoses for lesions of skeletal myopathy in turkeys include: exertional myopathy[5], nutritional myopathy associated with Vitamin E/ selenium deficiency, and toxic myopathy caused by ingestion of ionophores or toxic plants such as *Senna occidentalis*[3]. Based on a strong index of suspicion for ionophore toxicosis, feed samples were submitted for testing, revealing 36 ppm of the ionophore salinomycin.

Several ionophores including monensin and lasalocid are approved for use in growing turkeys in Canada as an aid in the prevention of coccidiosis caused by *Eimeria adenoides*, *E. meleagrimitis* and *E. gallopavonis*. However, salinomycin, although approved for use in chicken broilers, is excluded from this list as it is known to be toxic to turkeys even at very low levels. A caution on the Canadian salinomycin label reads: "Do not allow turkeys, dogs or horses access to this drug. It is known to be toxic to these species. Extra care should be taken to avoid contamination of feeds for these animals[2]". We have no further details as to how and when the salinomycin-contaminated feed was introduced to this specific flock of breeding turkeys, however, in most cases, there is a feed mill or on-farm feed mixing error, or salinomycin-medicated broiler feed is mistakenly fed to the turkeys.

Salinomycin can rapidly cause high mortality in affected flocks. In one recent case report, feed containing salinomycin was given to 13.5 weeks-old turkeys on May 29th. On June 2nd, the error was discovered and the salinomycin-medicated feed was removed but by June 3rd, more than 60% of the total mortality of 34.5% had already occurred[6]. The producer in the current case presented here lost almost all of the turkeys in this breeder flock as a result of ionophore toxicosis. Typical clinical signs include depression, stiffness, weakness, recumbency with extended legs, paralysis and death. The live turkeys in this case were described as panting and panting or dyspnea has been also been previously noted in turkeys with ionophore toxicosis[1]. Microscopic changes are reported in skeletal muscle and in some instances heart. Birds with respiratory signs often have lesions in tracheal muscles[3], and these were noted on histology in this case, although lesions in the myocardium were minimal. Ionophores interfere with ion transfer at the cell membrane, facilitating movement of K+ ions out of myocytes and increasing Ca++ uptake. Toxicity is a result of skeletal muscle damage; type I fibers appear to be selectively affected[3]. Ionophore toxicity is dose dependent, species and age-dependent, and simultaneous use of other drugs may also augment toxicity in some cases[1].

Evaluation of serum CK levels can be helpful in providing rapid confirmation of a skeletal myopathy when dealing with field



Skeletal muscle, turkey. Throughout the section, randomly scattered groups of myofibers are swollen, vacuolated, and exhibit contraction bands (degeneration and necrosis). (HE, 256X)

cases of recumbency in turkeys, as differential diagnoses include botulism[4].

JPC Diagnosis: Skeletal muscle: Degeneration and necrosis, multifocal, moderate, *Meleagris gallopavo*, avian.

Conference Comment: In domestic animals, toxic myopathies are generally caused by ingestion of ionophores, toxic plants, and plant-origin toxins. As a metabolically active organ system, skeletal muscle is highly susceptible to toxic injury resulting from membrane damage, altered protein synthesis, increased intracellular calcium concentration, or mitochondrial damage. Cases of myotoxicosis have variable clinical signs including: elevated serum concentrations of skeletal muscle cytoplasmic enzymes (CK and AST); severe muscle pain with or without myoglobinuria; or severe muscle weakness, recumbency and myoglobinuria. Animals often die from damage to cardiac muscle from the same toxin.[3]

Ionophores such as monensin, lasalocid, salinomycin, narasin, and maduramicin are compounds that alter membrane permeability to electrolytes by influencing transmembrane transport and function at low concentrations as a coccidiostat in birds and other animals. Monensin is the most common cause of ionophore toxicosis, and is produced by the fermentation of *Streptomyces cinnamonensis*. In addition to its function as a coccidiostat, monensin also promotes growth in ruminants. Toxicity results when animals are fed high concentration rations due to mixing errors,

or when fed to monogastric animals that have a reduced tolerance to the drug; when medications are added to rations (tiamulin, triacetyloleandomycin, or sulfonamides) the toxic effects of ionophores are potentiated. Maduramicin is another ionophore antibiotic and a common coccidiostat in poultry that has demonstrated cardiotoxicity in cattle and sheep. With ionophore toxicity clinical signs can vary; however, with the administration of large single doses, birds may present with lethargy, stiffness, muscular weakness, and recumbency within 24-hours of ingestion. Toxic effects are cumulative with the ingestion of smaller doses, culminating in myocardial lesions and cardiac failure within 2-3 weeks. Myocardial lesions predominate in horses, in contrast to sheep and pigs where skeletal muscle damage with myoglobinuria is more prevalent. . In cattle, both skeletal and cardiac muscles appear equally affected. Microscopically, ionophore toxicity is characterized by multifocal monophasic necrosis of both types 1 and 2 muscle fibers with macrophage infiltration within 48 hours of ingestion; this differs from nutritional myopathies which cause polyphasic necrosis. Ultrastructurally, mitochondria are swollen and degenerate due to disruption of the membrane transport of sodium and potassium, leading to increased intracellular calcium and mitochondrial failure.[3]

Common plants that result in toxic myopathy are: *Cassia occidentalis* or *C. obtusifolia* (senna or coffee senna beans) and *Karwinskia humboldtiana* (coyotillo). Similar to ionophores, ingestion of these plants leads to weakness and eventual recumbency with skeletal muscle pallor, and microscopic multifocal monophasic myonecrosis.[3]

Gossypol is a polyphenolic substance found in cottonseeds (*Gossypium* spp.) that is toxic to most domestic animals (particularly, swine), causing lesions in several organs including the heart, skeletal muscle, liver, and lung. Similar to the aforementioned toxins, death is due to cardiac failure and monophasic myonecrosis; also, there is hepatic centrilobular necrosis and pulmonary edema.[3]

Additionally, the following plants have been known to cause toxic myopathy in select species: *Diaporthe toxica* (lupinosis in sheep), *Cicuta douglasii* (sheep with water hemlock), *Thermopsis montana* (calves with false lupine), *Ageratina* spp. (horses and ruminants with white snake root), *Isocoma pluriflora* (rayless goldenrod), *Acer negundo* (horses ingesting hypoglycin A found in the box elder tree), and selenium toxicosis in pigs, cattle, sheep and other domestic species.[3]

Unless there is known history of ingestion or the presence of the toxin in the intestinal tract, these toxic myopathies are impossible to differentiate.

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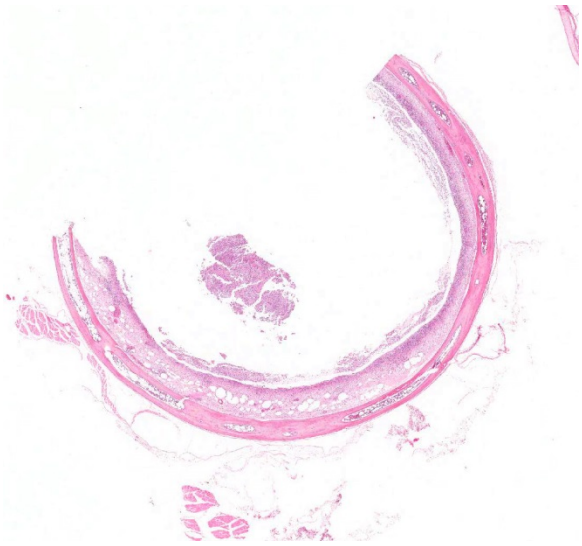


Trachea, peacock. There is diffuse circumferential loss of mucosal epithelium and expansion of the underlying submucosa by a cellular infiltrate and edema. A fibrinonecrotic membrane covers the denuded mucosa and there is a plug of necrotic exudate in the lumen. (HE, 6X)

CASE IV: T17-14880 (JPC 4101090).

Signalment: Adult, peacock, *Pavo cristatus*, avian.

History: The bird and others in the same flock had respiratory symptoms with rapid breathing and purulent ocular discharge.

Gross Pathology: Pasty to dry exudate was present on the conjunctiva of the eyes. Upon opening the carcass, the bird was found in a fair body condition. The oropharynx was rough and crusty. The crop was empty. Liver, kidney and lungs were congested. There was no other grossly visible lesion.

Laboratory results: Oropharyngeal/tracheal tissues were positive for herpesvirus by PCR. The sequenced amplicon showed identity to Gallid herpesvirus-1.

Microscopic Description: Trachea and larynx: The lesions vary from section to section. In all sections the tracheal mucosa is variably infiltrated with abundant lymphocytes, macrophages, scattered multinucleated syncytial cells and heterophils. The inflammatory cells trans-migrate the epithelium and also extend to the submucosa. Scattered epithelial cells contain eosinophilic intranuclear inclusions. The laryngeal mucosa (sections not included) is ulcerated and large numbers of lymphocytes, macrophages, plasma cells and occasional heterophils infiltrate the submucosa. Large numbers of lymphocytes, plasma cells and scattered heterophils are also observed in the submucosa of palpebral conjunctiva (sections not included). In a focally extensive area, abundant lymphocytes, macrophages and several multinucleated giant (syncytial) cells infiltrate a section of bronchus (sections not included), extend to the adjacent pulmonary parenchyma and variably fill the parabronchus and air capillaries. Scattered epithelial cells contained eosinophilic intranuclear inclusions.

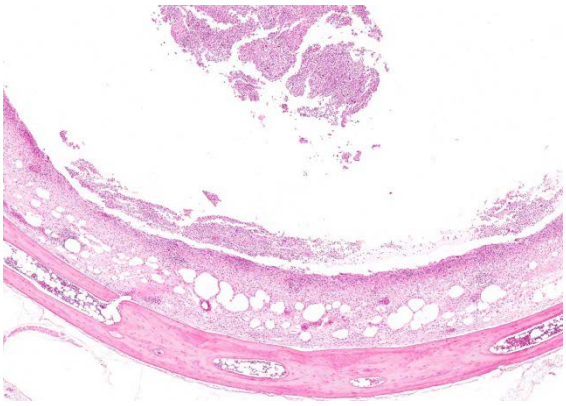
Contributor's Morphologic Diagnosis:

Trachea and larynx: Lymphoplasmacytic and heterophilic to pyogranulomatous tracheitis and laryngitis (laryngotracheitis) with syncytial cells and intranuclear inclusion bodies.

Contributor's Comment: The lesion is consistent with infectious laryngotracheitis due to gallid herpesvirus-1. Infectious laryngotracheitis (ILT) is a highly contagious respiratory disease of poultry reported in most countries around the world and causes significant economic losses in the poultry industry worldwide.[1, 4, 7] ILT virus (ILTV) belongs to alphaherpesviridae and the Gallid herpesvirus-1 species.[7] Different strains of the virus are recognized. In US infectious laryngotracheitis virus (ILTV) strains and field isolates were genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) into nine different genotypes.[5]

Natural transmission of ILTV is via respiratory and ocular routes. All ages of chickens including pheasants, pheasant-bantam crosses, and peafowl are affected, but chickens older than 3 weeks are most susceptible. The sources of ILTV are clinically affected chickens, latent infected carriers, contaminated dust, litter, beetles, drinking water and fomites.[7] The ILT virus can be spread by the transportation of animals, personnel, and equipment. In one study it was indicated that one of the critical points identified as a potential source of virus transmission was roads that were frequently used by the poultry industry within the outbreak area.[6]

Clinical signs can be severe or mild. Dyspnea and bloody mucus and high morbidity and mortality could be seen in severe forms. In the mild form depression, reduced egg production and weight gain, conjunctivitis, swelling of the infraorbital



Trachea, peacock. Higher magnification of the trachea. The outer ring of bone may be seen in sections taken close to the larynx. (HE, 62X)

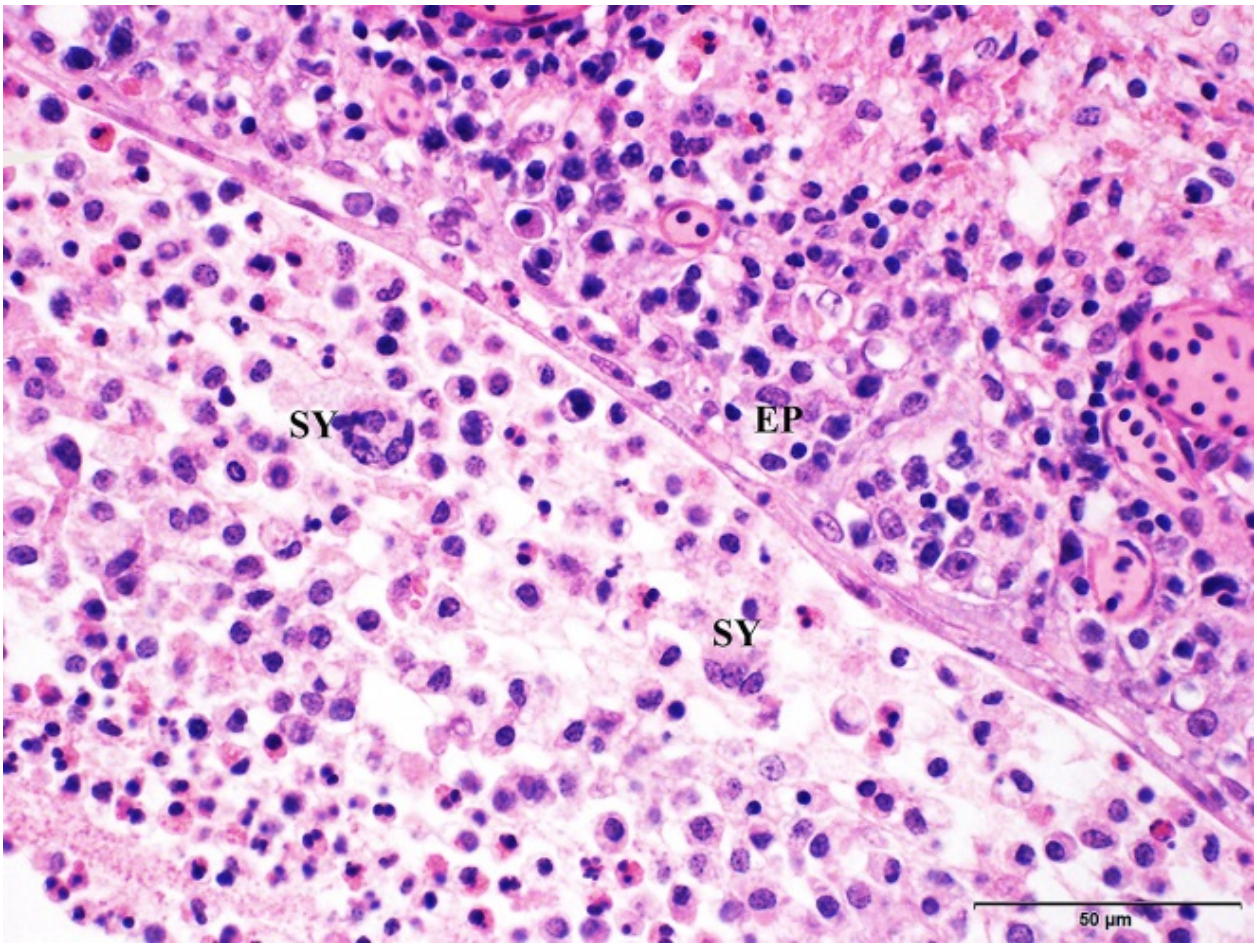
sinuses (almond shaped eyes), and nasal discharge are observed. The mild form is the most commonly seen type in the US and is called "silent, vaccinal, or almond-shape eye" ILT.[7] Clinical cases with the history of the pump handle type of respiration, conjunctivitis, coughing up of blood and mortality up to 80% is often reported by poultry farmers and veterinarians in India.[2]

Gross lesions are observed in the larynx and trachea. With the severe form, the mucosa of the respiratory tract shows inflammation and necrosis with hemorrhage. A characteristic feature is intranuclear inclusion bodies in epithelial cells. Inclusion bodies are generally present for a few days at the early stage of infection before epithelial cells die. Epithelial cells also form multinucleated cells (syncytia). Laboratory diagnosis (virus isolation and DNA detection) is required to confirm ILT, and rule out other diseases such as infectious bronchitis, Newcastle disease, avian influenza, infectious coryza, and mycoplasmosis that may have similar clinical signs and lesions.

Vaccination is effective to prevent ILTV infection. However, vaccine viruses can create latent infected carrier, which could be a source for spread of virus to non-

vaccinated flocks. Therefore, it is recommended that ILT vaccines be used only in endemic areas. It is important to avoid contact between vaccinated or recovered field virus infected birds with non-vaccinated chickens. It is also critical to remove contaminated fomites for prevention and control of ILTV infection. To control ILTV outbreaks, improved biosecurity and management practice are necessary.[7] Previous studies have demonstrated that the most effective way to prevent or control an ILT outbreak is through enhanced biosecurity, and implementation of an appropriate vaccination program.[6] However, although vaccination may have an important role in controlling ILT in outbreak areas, problems may occur when vaccine is administered incorrectly, vaccination fails to provide immunity to most birds in a flock, and biosecurity measures fail to prevent spread of vaccine virus to unvaccinated flocks.[3]

Recombinant viruses which possess significantly higher virulence and replication capacity were found to emerge as a result of recombination between live vaccine strains



Trachea, peacock. The necrotic membrane is composed of numerous sloughed mucosal epithelium, admixed with heterophils, and polymerized fibrin. The mucosa is covered by attenuated epithelium. The luminal exudate contains occasional syncytial cells (SY). EP= tracheal Epithelium. (HE, 400X) (Photo courtesy of: The University of Georgia College of Veterinary Medicine, Department of Pathology,
Tifton Veterinary Diagnostic & Investigational Laboratory, Tifton, GA 31793, http://www.vet.uga.edu/dlab/tifton/index.php)

(SA2 and A20), and another live vaccine strain (Serva) introduced into Australia in 2007. Interestingly, many of the ILT outbreaks occurred in vaccinated flocks. It is possible that these cases resulted from an inadequate vaccine dose or improper vaccine handling or administration.[1] Replication and spread of vaccine viruses is potentiated by vaccine administration that fails to provide immunity to all the birds in a flock (e.g., via drinking water) and when biosecurity measures fail to prevent spread to unvaccinated flocks.[3]

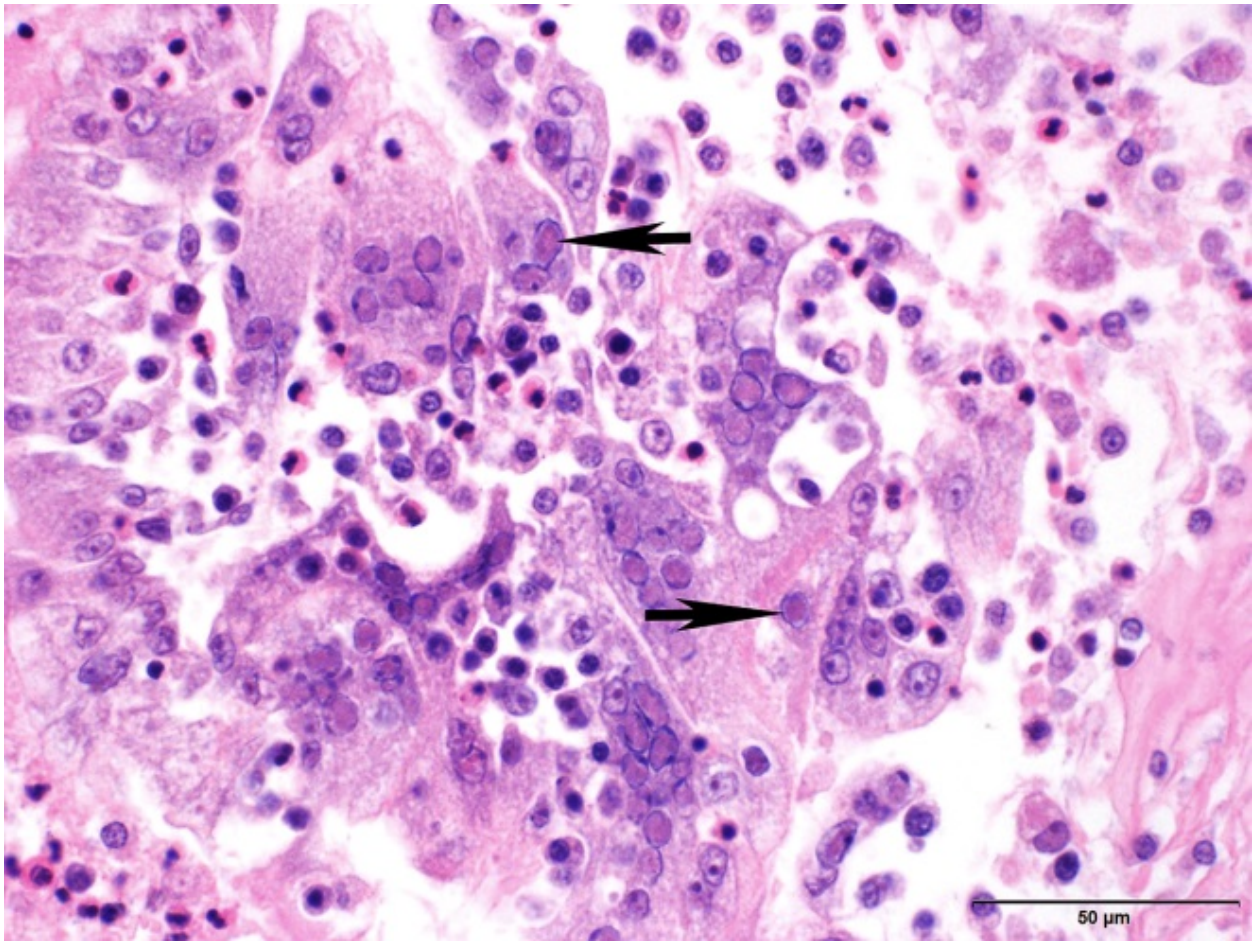
JPC Diagnosis: Trachea: Tracheitis, necrotizing and lymphohistiocytic, circumferential, severe with multinucleated viral syncytia and intranuclear eosinophilic viral inclusions, *Pavo cristatus*, avian.

Conference Comment: Several gross differentials were discussed in the conference, including: infectious bronchitis, Newcastle disease, avian influenza, infectious coryza, and mycoplasmosis.

Infectious bronchitis (IBV), caused by a coronavirus, occurs naturally in chickens of all ages. IBV has many different serotypes and with the current vaccine there is little cross-protection between serotypes compounding that with a high mutation rate makes this disease difficult to control and diagnose. Gross lesions include upper respiratory tract infections with or without airsacculitis. Some serotypes affect the kidneys (nephrotropic strains) and can result in swollen kidneys (interstitial nephritis histologically) with uric acid crystals in renal tubules and ureters. Microscopically, the tracheitis is characterized by mucosal edema, cilia loss, degeneration and necrosis of mucosal epithelial cells, and inflammation.[6]

Exotic Newcastle disease (ND), caused by avian paramyxovirus-1 (APMV-1), occurs most commonly in chickens and less often in turkeys (although most poultry are susceptible) of all ages. There are three main strains of APMV-1: (1) Lentogenic strains which are mildly pathogenic strains; (2) Mesogenic strains which are moderately pathogenic; and (3) Velogenic strains which are markedly pathogenic strains. Usually, "enzootic" strains of ND are lentogenic or mesogenic and result in mild respiratory infections with occasional nervous signs (abnormal positions of the head and neck, AKA "star gazers", paralysis, prostration). Velogenic strains cause two main pathotypes: (1) Neurotropic velogenic which cause respiratory and nervous signs with high mortality and (2) Viscerotropic velogenic which cause hemorrhagic intestinal lesions with high mortality. With velogenic strains, gross lesions can be diverse and may consist of one or more of the following: diphtheritic laryngotracheitis; conjunctival hemorrhage; air sacculitis; facial edema with hemorrhage of the comb and wattles; hemorrhages on the mucosa of the proventriculus or gizzard, Peyer's patches, cecal tonsils, and large intestine; multifocal splenic necrosis; egg yolk in the abdominal cavity; and deformed eggs.[6]

Avian influenza (AIV) is caused by a type A influenza virus in the Orthomyxoviridae family which has two important surface antigens which help in identification and subtyping of the virus: hemagglutinin (H) which aids in viral attachment and neuraminidase (N) which cleaves sialic acid residues on host cells and mediates virion release. There are many different strains of influenza that are classified into two categories: low pathogenic (LPAI, cause little to no clinical signs) and high pathogenic (HPAI, which cause severe clinical signs and high mortality).



Parabronchus, peacock. The parabronchus contains numerous syncytial cells with multiple nuclei, many of which contain a single prominent eosinophilic herpesviral intranuclear inclusion. (HE, 400X) (Photo courtesy of: The University of Georgia College of Veterinary Medicine, Department of Pathology, Tifton Veterinary Diagnostic & Investigational Laboratory, Tifton, GA 31793,
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Additionally, HPAI is subcategorized if they are highly pathogenic and "notifiable" strains (HPNAI). Wild reservoirs are waterfowl and shorebirds that are commonly asymptomatic and excrete the virus in their feces for many years. Once introduced into a farm, AIV is transmitted by direct and indirect contact through respiratory secretions and excrement and can be transferred from farm to farm on fomites. Gross lesions associated with LPAI outbreaks include: mild tracheitis, sinusitis, air sacculitis, and conjunctivitis and fibrino-purulent bronchopneumonia can occur with secondary bacterial infections. With HPNAI outbreaks, gross lesions are generally more severe to include: fibrinous exudates on airsacs, in the peritoneum, or pericardial sac; multifocal areas of necrosis externally on the skin, comb, and wattles or internally on the liver, kidney, spleen, or lungs, blotchy, red discoloration of the shanks, hemorrhage and petechiae on mucosal and serosal surfaces of the proventriculus and gizzard. In turkeys, encephalitis and pancreatitis have been reported as well.[6]

Infectious coryza, caused by *Avibacterium paragallinarium*, primarily affects chickens but has been rarely reported in pheasants

and guinea fowl with upper respiratory tract infections often complicated by other agents like *Mycoplasma gallisepticum* which leads to chronic respiratory disease. Transmission of infectious coryza probably occurs through inhalation of infectious droplets or ingestion of infected feed materials. *A. paragallinarium* cannot exist long outside the host and is easily eliminated by disinfectants or environmental extremes. Typical gross lesions are catarrhal inflammation in the sinuses with nasal discharge, conjunctivitis with caseous exudate, edema of the face and wattles, and tracheitis, pneumonia, or air sacculitis in cases complicated by secondary pathogens.[2]

Mycoplasma gallisepticum is the causative agent of chronic respiratory disease in chickens and infectious sinusitis in turkeys. It is initially transmitted transovarially and can then be spread by aerosol transmission to other chicks or through contaminated feed. As with all mycoplasma infections, clinical signs take time to develop and show more profound lesions in broilers 4-8 weeks old. Gross lesions are similar to the other diseases listed above and include: catarrhal inflammation of the sinuses and upper airways, airsacculitis with hyperplastic lymphoid follicles, fibrinous perihepatitis, and adhesive pericarditis.[2]

Due to the similarities in gross and microscopic findings among the diseases discussed above, laboratory diagnosis (virus isolation and DNA detection) is required for definitive diagnosis.

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