Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 11

8 December 2021

CASE I:

Signalment:
2-week-old chicken (*Gallus gallus*)

History:
A 2-week-old specific pathogen free chicken was subcutaneously inoculated with infectious bursal disease (IBD) virus which is classified as a very virulent virus. The present case exhibited ruffled feathers on day 3 postinoculation. The bird showed severe depression and was found dead on day 4 postinoculation.

Gross Pathology:
The bursa of Fabricius (BF) was hemorrhagic and slightly enlarged. The muscle of both legs exhibited focal hemorrhages. The hemorrhage was also found at the papillae of the mucus membrane of the proventriculus. The thymus was yellowish, and was severely atrophied. The kidney was pale.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
There was a severe depletion of lymphocytes in the bursa (karyorrhexis), remaining follicular epithelial cells, and foamy macrophages. Severe hemorrhage and many fibrin thrombi were observed in the interstitium. Bacterial colonies observed in the section were considered to have occurred secondary or postmortem. Viral antigens were detected in cells of the lymphoid follicles and interstitium by immunohistochemistry.

Contributor's Morphologic Diagnoses:
Bursa of Fabricius: Diffuse lymphoid necrosis with macrophage infiltration, hemorrhage, fibrin thrombi. (experimental case of IBD)

Contributor's Comment:
IBD is a highly contagious viral disease of chickens.[5,12] IBD, also known as Gumboro disease, is caused by IBD virus of the family

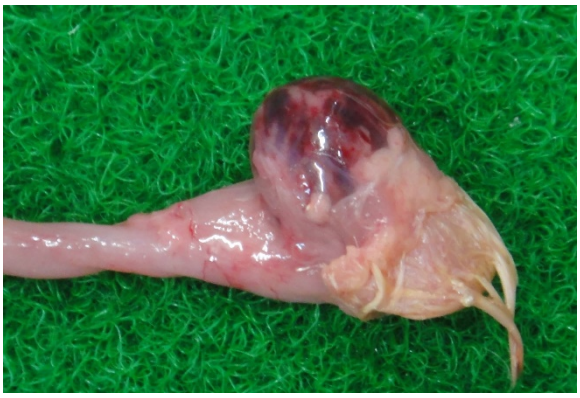

Figure 1-1. Bursa of Fabricius, chicken. The bursa of Fabricius (BF) is hemorrhagic and slightly enlarged. (*Photo courtesy of:* National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, http://www.naro.affrc.go.jp//english/niah/index.html)

Birnaviridae.[5,12] The genome of IBD virus consists of two segments (A and B) of double-stranded RNA.[5,12] The genome encodes 5 viral polypeptides (VP1-5) .[5] The large segment A encodes VP2-5; the smaller segment B encodes VP1.[5] VP2 and VP3 are the major structural proteins of the virion.[5] VP2 is credited with eliciting protective immunity in birds.[5]

Two serotypes of IBD virus are recognized.[5,12] The IBD viruses of serotype 1 are pathogenic for chickens. The serotype 2 viruses are nonpathogenic. The IBD viruses of serotype 1 are generally classified as classical virulent virus, very virulent virus, and antigenically variant virus. The very virulent virus was first identified during the 1980s, and has been detected in many countries.[11] Some phylogenetic analyses performed on very virulent viruses showed that they constitute a specific cluster in the phylogenetic tree.[11,12,13]

The infection of classical virulent viruses is mild or subclinical. However, the impaired growth and immunosuppression as the result of infection can result in the secondary

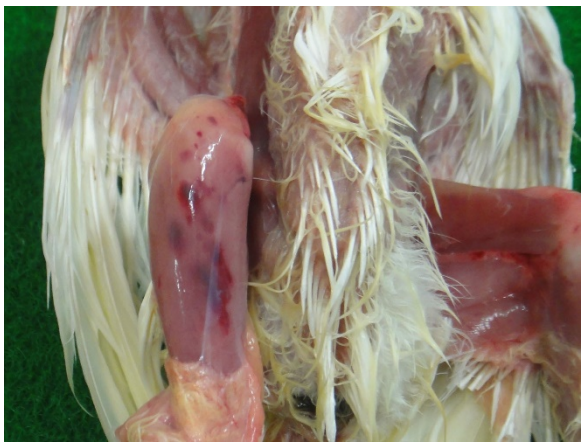

Figure 1-2. Skeletal muscle, chicken. There is multifocal hemorrhage within the skeletal muscles of the legs. (*Photo courtesy of:* National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, http://www.naro.affrc.go.jp/english/niah/index.html)

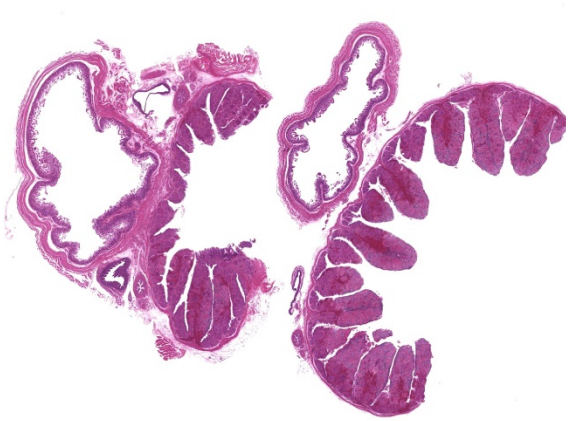

Figure 1-3. Bursa of Fabricius, chicken. Section of the bursa and adjacent rectum are submitted for examination. The bursa is shrunken and atrophic, with marked lymphocyte depletion. (HE, 7X)

infection and vaccination failure. The very virulent viruses have the ability to cause high mortality rate in affected flocks (i.e. 50-60% mortality in laying hens). The principal macroscopic lesions in chickens exhibiting clinical signs are the edematous swelling, discoloration, and hemorrhage of the bursa.[5] Hemorrhages in the pectoral muscles and thigh muscles can be observed frequently.[5,11,12] This may be caused by a coagulation disorder during the infection.[6]

Young immature chickens with active lymphocyte development are very susceptible to IBD. Under natural conditions, the infection appears to be via the oral route.[5] From the gastrointestinal tract, the virus is transported to other tissues by phagocytic cells, most likely macrophages.[5] The bursa of Fabricius is the most susceptible organ.[5] IBD virus infection occurs in B lymphocytes in the immature stage with immunoglobulin M expressed on the cell surface, leading to the cytolytic destruction of infected cells.[2] Viral antigens can be detected by immunohistochemistry in affected lymphoid follicles.[5,9] Some studies indicated that macrophages can be infected with IBD virus.[3,4] Although T lymphocytes are considered resistant to infection, the

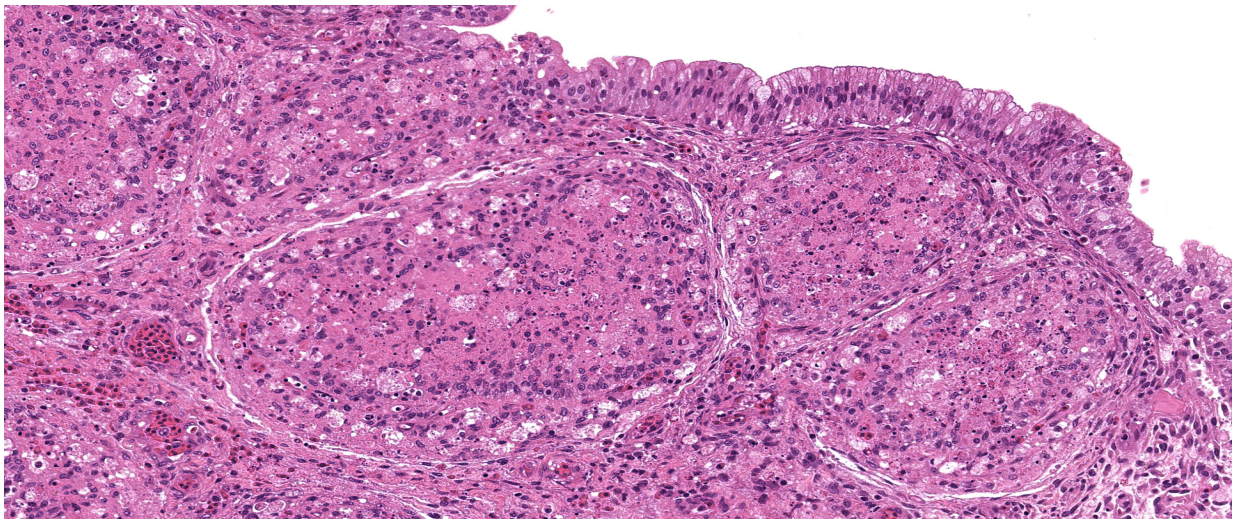

Figure 1-4. Bursa of Fabricius, chicken. There is total loss of lymphocytes within the bursal follicles, and the follicles are replaced with abundant cellular debris and debris laden macrophages. There is multifocal vacuolar degeneration of overlying bursal epithelium. (HE, 279X)

infiltrating T lymphocytes in the lesions may be involved in the pathogenesis.[2,5] The pathological lesions can be observed in other lymphoid organs including the thymus, spleen, cecal tonsil, and bone marrow.[9] Thymic atrophy may be associated with extensive apoptosis of thymocytes during the acute phase of virus infection.[8]

Vaccination is popular for protection against IBD.[12] Some live vaccine strains can cause mild to severe lesions to the BF, which may provoke immunosuppression and secondary disease.[12]

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JPC Diagnosis:
Bursa of Fabricius: Lymphoid depletion, follicular, diffuse, severe, with lymphocytolysis and hemorrhage.

JPC Comment:
First reported in Gumboro, Delaware in 1962, infectious bursal disease virus (IBDV) is a widespread pathogen of worldwide economic relevance. As described by the contributor, IBDV has historically been differentiated into two antigenic groups, serotypes 1 and 2, with serotype 1 strains further differentiated into classic and variant subtypes such as the very virulent strain.[7,11] However, significant genotypic and pathotypic variability exists amongst subtypes as the result of frequent mutations and recombination events.[7] Consequently, a new genogroup classification scheme based on the hypervariable region of the outer capsid protein VP2 (*hvVP2)* gene has been proposed, with seven genogroups (G1-G7) identified. The classic, variant, and very virulent strains comprise G1, G2, and G3, respectively.[7,11] Strains G4-G7 are composed of a fourth "distinct" strain (G4), a proposed group of recombinant Mexican strains (G5), and divergent strains predominantly identified in Italy (G6) and Australia (G7).[11] G4 is composed of several highly divergent strains reported in North and South America, Asia, and Europe with a unique genetic profile that cause subclinical infection with

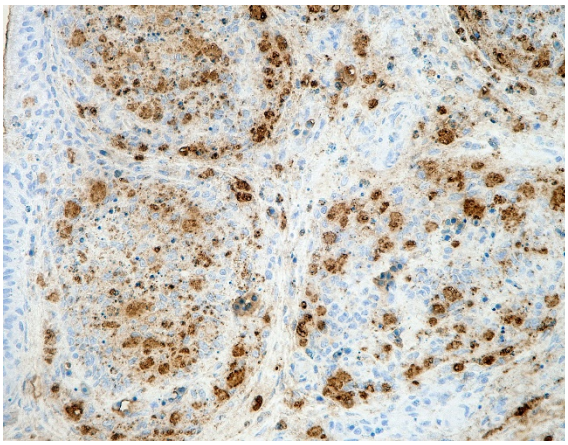

Figure 1-5. Bursa of Fabricius, chicken. Follicular remnants and the interstitium contain abundant birnaviral antigen. (*Photo courtesy of:* National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, http://www.naro.affrc.go.jp/english/niah/index.html)

bursal atrophy and immunosuppression. Information in regard to the genetic characteristics of the "distinct" strains of IBDV have historically garnered little attention due to lack of specific clinical signs but have recently come under increased scrutiny as the result of their prevalence and potentially significant economic impact, particularly in South America.[11]

As noted by the contributor, the virus predominately replicates in IgM-positive B lymphocytes, particularly in the bursa of Fabricius, in addition to other lymphoid tissues. The virus causes infected B lymphocytes and adjacent antigen-negative cells to both undergo apoptosis, leading to B cell depletion in the bursa. This process appears to be highly regulated as apoptosis is inhibited by VP5 early in the viral replication cycle, yet VP5 and VP2 induce apoptosis near the end of the replication cycle.[1] This phenomenon has also been reported to occur following the administration of vaccines containing live IBDV, with bursal damage occurring 7 to 35 days post vaccination depending on the strain. Notably, the onset of these lesions correlates with the initiation of antibody production. Risk of vaccine induced immunosuppression is a concern, particularly in regard to the potential for degraded humoral responses for other commonly vaccinated diseases, such as Newcastle disease. Conflicting information has been reported in regard to suppression the humoral response to other vaccinations when co-administered with IBDV, with results ranging from no effect on antibody titers despite lesion development in the bursa to significantly decreased antibody levels for three antigens, including Newcastle disease.[1]

The moderator discussed differentials for bursal atrophy or necrosis, which include chicken anemia virus, chronic Marek's disease, and Newcastle disease virus.

References:

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CASE II:

Signalment:
6-week-old male turkeys (*Meleagris gallopavo*)

History:
Grower facility in the Midwest with flock of 21,696 six-week-old Nicholas commercial tom turkeys. Birds were sourced from a single source parent flock and the hens from which the poults originated were in their 5th week of lay. Grower had over 5-year history of No Antibiotics Ever™ (NAE), however had recently switched to conventional production. The birds were started on lasalocid (coccidiostat), did well through the brooder (first 4 weeks) and adjusted well to the finisher, typically a stressful transition. The birds were vaccinated against hemorrhagic enteritis at approximately 17 days and boosted at 27 days of age. The birds became severely depressed with ruffled feathers and overt signs of discomfort. Water consumption was diminished and the birds exhibited moderate flushing (diarrhea). The grower did not report any respiratory signs. Mortalities increased from 3-5/day to 60-70 birds expiring daily. The grower was advised to revaccinate for hemorrhagic enteritis and start amprolium. The consulting poultry veterinarian was summoned to the site and necropsied multiple birds.

Gross Pathology:
The intestinal tracts were distended, dark purple and filled with hemorrhagic contents and the intestinal mucosa was covered by a diphtheritic membrane. The spleens were enlarged with pale white mottling.

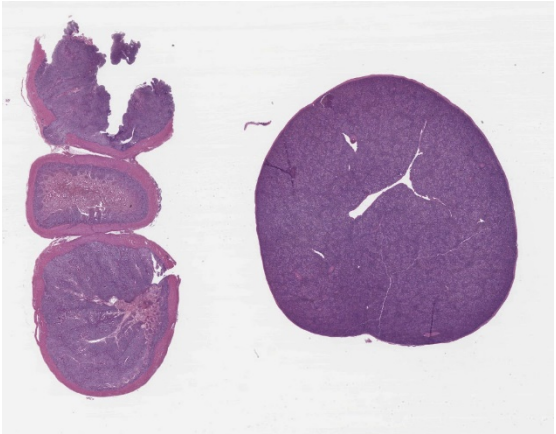

Figure 2-1. Cecum, intestine, spleen, turkey. The spleen (right) is markedly enlarged and there is loss of the splenic architecture. (HE, 6X)

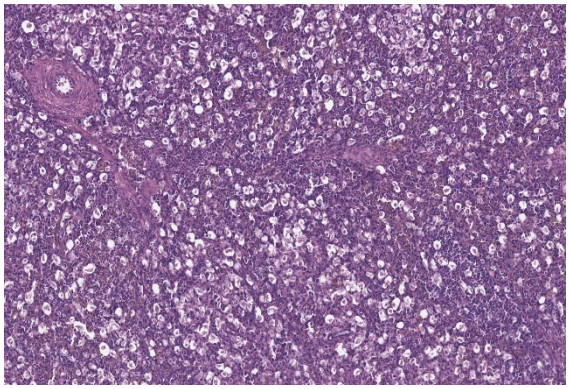

Figure 2-2. Spleen, turkey. Numerous macrophages contain cellular debris within their cytoplasm, creating a "starry sky" appearance. (HE, 250X)

Laboratory Results:
Anaerobic Culture, Intestine: *Clostridium perfringens*, heavy growth
Aerobic Culture, Intestine: *Salmonella* sp. serotype *infantis*
Fecal Float: *Eimeria*, few

Microscopic Description:
Small intestine: In one section, villar architecture is obscured by hemorrhage diffusely expanding the lamina propria. Other tissue sections depict simultaneous coagulative necrosis of villar tips with retention of villar outlines or partial to transmural mucosal ulcerative necrosis occasionally reaching the submucosa, mixed inflammatory reaction primarily lympho-histiocytic and heterophilic, intranuclear inclusions in the infiltrating lympho-histiocytic cells and gram-positive rods colonizing the villus tips and areas of necrosis. There is widespread villous epithelial necrosis and sloughing into the lumina with villous atrophy, laminal proprial collapse, hemorrhage and fibrinous exudate admixed with myriad protozoal oocysts (*Eimeria* sp.) Oocysts have a thick wall and centrally located nucleus.

Ceca: Villar tips are lined by swollen or exfoliating pyknotic nuclei with attendant hemorrhage, fibrinous exudation, widespread necrotic destruction, and effacement of cecal tonsillar architecture by histiocytic infiltrate. Lymphohistiocytic cells in the laminal propria contain intranuclear inclusion bodies marginating nuclear chromatin. There is concomitant intense intra-epithelial parasitization by the various developmental stages of *Eimeria* sp. eliciting widespread destruction of villous and cryptal architecture with surface epithelial necrosis, erosion, ulceration and fibrin exudation, squamous metaplasia of regenerative surface epithelium, mucosal collapse, and infiltration of the lamina propria by myriad heterophils. There is bacterial invasion and colonization of surface epithelium.

Spleen: There is widespread tissue pallor associated with necrosis and involution of white pulp and massive histiocytic infiltrate emanating from the sheathed arteries. Reticuloendothelial cells and infiltrating histiocytes contain large amphophilic to basophilic intranuclear inclusions with attendant chromatin margination characteristic for adenovirus and there are myriad tingible body macrophages imparting a "starry sky" appearance.

Contributor's Morphologic Diagnoses:
1. Spleen: Severe diffuse histiocytic splenitis with lymphoid depletion and intrahistiocytic intranuclear inclusions.

2. Small intestine/Cecum: Severe hemorrhagic and necrotizing enterotyphlitis with intra-lymphohistiocytic intranuclear inclusions, intraepithelial protozoa, and intralesional bacteria.

Contributor's Comment:
Adenoviral infection with attendant transient immunosuppression facilitating opportunistic bacterial and protozoal colonization underlies the constellation of macroscopic and histologic lesions and disease trajectory

in these birds. Adenoviral intranuclear inclusions visualized in lymphomononuclear cells in the intestinal and splenic tissues are characteristic for hemorrhagic enteritis (HE) of turkeys.

Hemorrhagic enteritis was first observed in 1936 in Minnesota turkeys ranging from 7-12 weeks of age and was described by Pomeroy and Fenstermacher.[1] Although the birds were well-nourished and in good flesh, clinically affected birds were depressed with ruffled feathers and had watery discharge emanating from the vent.[1] The entirety of the intestinal tract was visibly distended with mucosal congestion and serosanguineous intestinal contents, however microscopic lesions were most striking in the duodenum.[1] The principal early lesion involved erythrocytes and round cells infiltrating and thickening the lamina propria with the lesion progressing to exfoliation of villous epithelium and finally erosion and collapse of villous architecture.[1] Later, the causative agent underlying HE in turkeys and marble spleen disease (MSD) of pheasants and avian adenovirus splenomegaly (AASV) of broiler breeders was attributed to adenoviral infection.[2] HE of turkeys is associated with depression, splenomegaly, gastrointestinal hemorrhage, immunosuppression and death while MSD is primarily associated with respiratory disease in captive-raised pheasants.[2]

Utilization of adenoviruses as vectors for delivery of genes in vaccines and gene therapy prompted sequencing of the complete viral genome of the serologically indistinguishable 3 viruses. All 3 viruses were previously assigned to the genus *Aviadenovirus*, and designated as Group 2 aviadenoviruses.[3] Genomic differences amongst the 3 viruses were minor although genomic sequence homologies indicated they were sufficiently different from other aviadenoviruses to warrant reassignment to a new genus *Siadenovirus* based upon their highly conserved sequential homology to the bacterial genes encoding for sialidase.[4] Collectively these 3 viruses have been given the species name, Turkey adenovirus-3 (TAdV-3).[4] HEV is a linear double-stranded

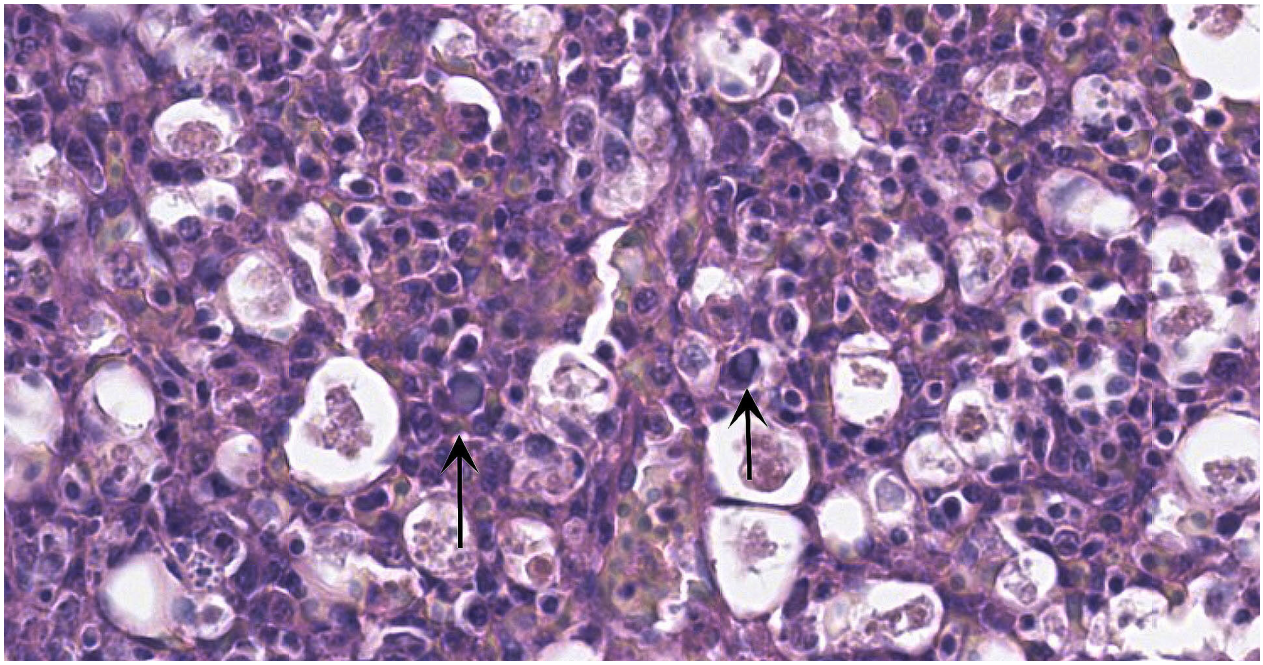

Figure 2-3. Spleen, turkey. Few histiocytes demonstrate karyomegaly with large basophilic intranuclear inclusions (arrows). (HE, 979X)

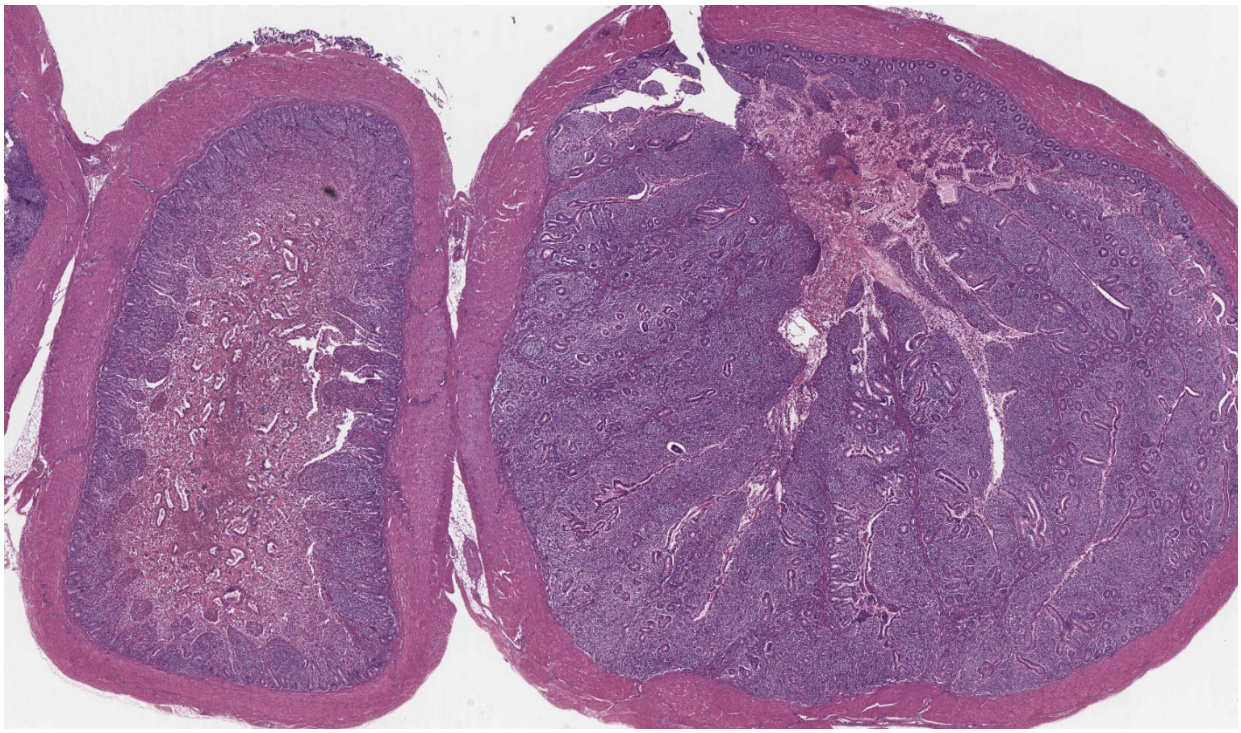

Figure 2-4. Small intestine (left), cecum (right), turkey: There is marked expansion of the lamina propria of the intestine and cecum by a cellular infiltrate. (HE, 46X)

DNA virus that is the smallest of any adenovirus thus characterized (26,263 bp) with low G+C content (34.93%).[3] The HEV genome contains 8 ORFs bearing no similarity to ORFs described for adenoviruses.[5] Several ORFs are arranged in two clusters-ORF 1,2,3,4 and ORF 7,8 with at least 13 genes encoding for structural proteins including the trimeric hexon, pentameric penton base, and trimeric fiber comprising the viral capsid.[5] These three structural proteins are produced in large amounts and appear to be the most antigenic.[6] Excepting minor genomic differences and host virulence factors, HEV and MSDV offer cross-protection directing vaccination strategies utilizing virulent MSDV strains in pheasants to vaccinate turkeys and strains of HEV virulent in turkeys to vaccinate pheasants.[6] Phenotypic differences in clinical disease and lesion severity are influenced by sequential variations in the ORF1, E3 and fib genes.[7]

Maternal antibodies generally prevent infection in birds less than 6 weeks of age with fecal-oral/cloacal drinking the most likely exposure route for natural disease.[8] To this end, prolonged environmental stability of viral particles in carcasses and wet fecal-contaminated litter mandate rigorous cleaning, disinfection and biosecurity practices.[8]

Upon oral exposure, absorbed viral particles target Ig-M bearing B-lymphocytes in the intestinal lamina propria and bursa of Fabricius, or enter the circulation homing towards the spleen, with viral replication targeting IgM^+ B-lymphocytes and macrophages together with influx of $CD4^+$ lymphocytes (acute infection) underlying white pulp hyperplasia and splenomegaly.[9] HEV replication in IgM bearing B-lymphocytes and macrophages induces apoptosis and necrosis and reduced antigen presentation by lymphocytes and macrophages, offering two possible mechanistic explanations for HEV-induced immuno-

suppression leading to diminished humoral immunity against opportunistic infectious agents and vaccine failure.[10,11] During peak infection, elaboration of Types I and II interferons and pro-inflammatory cytokines, namely IL-6 and TNF play competing protective and destructive roles.[10,11] Elaboration of pro-inflammatory cytokines presumably modulates hemorrhagic disease because treatment with TNF antagonists prevents HEV-induced intestinal hemorrhage and diapedesis.[10] The evolution of HEV-induced intestinal lesions owing to increased duodenal mast cells in response to cytokine elaboration by activated T cells is described.[12]

Given HEV tropism for IgM^+ B-lymphocytes with attendant immunosuppressive effects, TAdV-3 infected birds are predisposed to secondary colibacillosis and may facilitate development of necrotic enteritis.[13]

In this case, the grower added trimethoprim/sulfa in the water for 7 days. The birds greatly improved, resuming normal water consumption and activity. The grower expressed concern about the viability of the initial HE vaccine. The vaccine should arrive frozen and stays frozen until thawed and mixed with a stabilizer over a 4 hour period. The grower indicated the 125,000 dose shipment and surrounding ice packs arrived already thawed. The vaccine was re-frozen and thawed again to be administered at both 17- and 27-day vaccines suggesting vaccine failure and inadequate protection.

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JPC Diagnosis:

1. Spleen: Splenitis, histiocytic, diffuse, severe with intranuclear viral inclusions, lymphoid depletion, and lymphocytolysis.

2. Ileum and cecum: Enterotyphlitis, histiocytic and lymphoplasmacytic, diffuse, severe with intranuclear viral inclusions.

3. Ileum and cecum: Intraepithelial apicomplexan schizonts, numerous with intraluminal oocysts.

JPC Comment:
The contributor provides an excellent review of Turkey adenovirus-3, the etiologic cause of hemorrhagic enteritis (HE), an economically significant disease most commonly seen in turkeys greater than 4 weeks of age.[13]

Although Pomeroy and Fenstermacher first described HE in 1937, it wasn't until 1967 that Gross and Moore discovered the etiologic agent was filterable through a 0.22 micron filter, supporting speculation of a viral entity. This suspicion was confirmed in 1974, when Carlson et al. identified adeno-like viral particles in tissues from affected birds.[13]

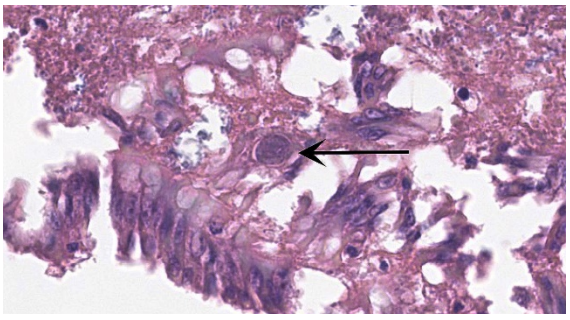

Figure 2-5. Small intestine, turkey: There are rare coccidian oocysts (arrow), within the lumen of the small intestine. (HE, 600X)

Introduction of HEV to susceptible flocks most often occurs as the result of exposure of naïve turkeys to contaminated feces or litter material. Lacking an envelope, TAdV-3 virons persist in the environment, retaining the ability to infect new hosts for up to 6 months at 4ºC and 4 years at -20ºC. In addition, virons are resistant to chemical inactivation by quaternary ammonium compounds, ethyl ether, and zepharin chloride. However, exposure of the virus to high temperatures (>70ºC) for one hour or treatment with other agents such as 1% Lysol, 1% sodium lauryl sulfate, or sodium hypochlorite after removal of all organic material renders it non-infective.[13]

Although TAdV-3's environmental persistence has been established as a source of subsequent infection, recent findings have suggested recovered birds may also serve as asymptomatic carriers.[13] Thus production facilities may risk re-contamination following post-outbreak sanitation measures if an affected flock is not depopulated.

As described by the contributor, apoptosis and necrosis of infected B-lymphocytes and macrophages results in impairment of both humoral and cell mediated immunity. Consequently, affected birds are not only more susceptible to secondary infection, but responses to vaccination are also suppressed, as has been demonstrated with low antibody titers following vaccination for Newcastle disease.[13]

Multiple entities cause similar gross splenic and gastrointestinal lesions in turkeys, including bacterial etiologies such as *E. rhusiopathiae*, *P. multocida, E. coli,* and *Salmonella sp.*, as well as viral entities including Newcastle disease, avian influenza, reticuloendotheliosis, and lymphoproliferative disease. Histologically, the presence of adenoviral nuclear inclusions in lymphocytes and macrophages is diagnostic although they may not always be present. Additional diagnostics tests include agar gel immunodiffusion, antigen-capture ELISA, immunohistochemical staining, and immunofluorescence or immunoperoxidase methods. In addition, quantitative real-time PCR is a highly specific and sensitive method that can be particularly useful in outbreak scenarios as large numbers of samples can be processed in less time than when using conventional methods.[13]

Conference participants noted significant variation between slides in regard to the number of intraepithelial apicomplexans and intraluminal oocysts present within tissue sections.

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CASE III:

Signalment:
Juvenile male Western crow (*Corvus brachyrhynchos hesperis*)

History:
This case is one in a series of juvenile crows that were part of a large mortality event at the San Diego Zoo Safari Park in the first few weeks of August, 2020. Most crows were found deceased on park grounds and submitted for necropsy.

Gross Pathology:
A juvenile male crow was presented for necropsy. The skin and subcutis were tacky and dry (dehydration). There were minimal to no fat stores, and the pectoral muscles were depressed below the keel (poor body condition). The intestine was segmentally dark purple to green and the ceca were bilaterally plump. The spleen was prominent and dark red.

Laboratory Results:
PCR (Frozen intestine and spleen from select cases): Approximately 50% of samples were positive for orthoreovirus using conventional PCR targeting the L2-genome segment encoding the RNA-dependent RNA-poly-merase.

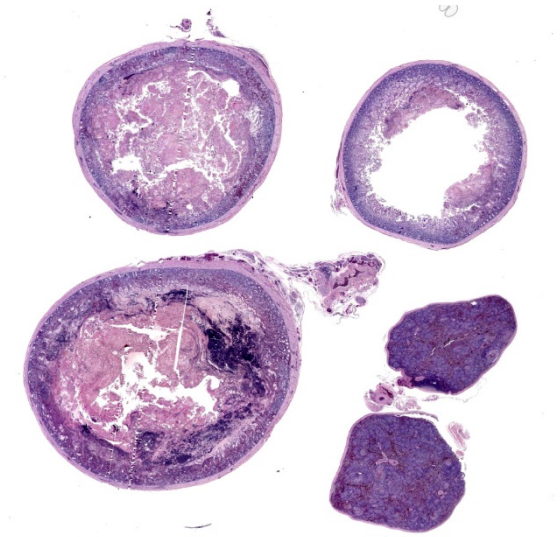

Figure 3-2. Intestine, colon, spleen, crow. Multiple sections of intestine (top), one section of colon (lower left), and two section of spleen (lower right) are submitted for examination. At subgross magnification, there is transmural loss of normal architecture of the intestinal and colonic mucosa. (HE, 5X)

Microscopic Description:
The slide contains two sections of small intestine, one section of large intestine, and one or two sections of spleen.

Intestine: All sections of intestine are similarly affected by segmental to circumferential necrosis, characterized by deep ulceration and loss of the mucosa, lamina propria, and submucosa with replacement by abundant, fibrillar eosinophilic material (fibrin), erythrocytes (hemorrhage), and fragmented cellular and nuclear debris admixed with large colonies of bacilli. In less affected segments, necrotic material forms a thick, intermittent pseudomembrane adherent to the remnant mucosal surface, and in more severely affected sections fibrinonecrotic debris fills the entire lumen. Crypts are dilated, distorted, and variably separated, and frequently contain clumps of necrotic cells and pale eosinophilic, flocculent debris. Occasionally, crypts contain small to moderate numbers of luminal and rarely intracellular spirochetes. Crypt epithelium ranges from piled and hypertrophic to flattened and attenuated. The intervening lamina propria is expanded by abundant erythrocytes, heterophils, histiocytes, and aggregates of fibrin, and lymphoid tissues are expanded by clear space and karyorrhectic debris (edema and lymphocytolysis, respectively). Lymphocytes, plasma cells, fewer granulocytes, and erythrocytes dissect within the muscular wall along mural vessels and multifocally extend to the serosa and adjacent mesentery, which is disrupted by aggregates of intact and degenerate leukocytes.

Spleen: The spleen is multifocally disrupted by foci of fibrillar, pale eosinophilic material (fibrin), erythrocytes, and smudged to stippled cellular and karyorrhectic debris (necrosis). Foci are variably associated with aggregates of foamy histiocytes, many of which contain intracytoplasmic, coarsely-granular, golden-brown, birefringent pigment (suspect hemozoin). There are abundant shrunken cells with fragmented and pyknotic nuclei throughout all sections (lymphocytolysis).

Contributor's Morphologic Diagnoses:
1. Small intestine, colon, ceca: Severe, multifocal to segmental, acute, fibrino-

Figure 3-1. Intestine, crow. The intestine and ceca are deep purple and diffusely necrohemorrhagic. (*Photo courtesy of:* San Diego Zoo Wildlife Alliance Disease Investigations, P.O. Box 120551, San Diego, CA 92112-0551, https://science.sandiegozoo.org/disease-investigations)

necrotizing, hemorrhagic, and heterophilic enterotyphlocolitis

2. Spleen: Moderate multifocal acute fibrinonecrotizing splenitis with mild birefringent pigment deposition (hemozoin, presumptive)

Contributor's Comment:
A large number of crows were submitted to Disease Investigations at the San Diego Zoo over a one month period through August of 2020, all of which were juveniles found dead or moribund. Consistent lesions included severe necrohemorrhagic enterotyphlocolitis, fibrinonecrotizing splenitis, and poor body condition. Additional, less consistent lesions (not submitted) included depletion of lymphocytes in the bursa of Fabricius, myeloid hyperplasia in the bone marrow, and acute renal tubular degeneration and necrosis (possibly dehydration-related). The findings are consistent with those attributed to avian orthoreovirus, which has been reported in American corvids as early as 2001 and was more recently described in a retrospective review of crow mortalities in New York.[2] Classically, outbreaks of the virus in crows have occurred during winter months, lending it the name "winter mortality of crows".[2] This particular event was unusual in that it occurred in late summer, however it appeared to be associated with large numbers of gathered crows based on subjective observation, which is a known prerequisite for epizootics.[2] Secondary bacterial infections (especially bacterial enteritis) and sepsis were common sequelae in many crows, and some cases had coinfections with hemoparasites, Aspergillus sp., and avian poxvirus.

Avian reoviruses are non-enveloped, double-stranded RNA viruses that are common in commercial poultry, where disease is typically manifested as tenosynovitis.[1,4] The virus has been classified into six genotype clusters based on the sigma C protein sequence, which demonstrates the greatest variability between viral isolates.[1] Thus far,

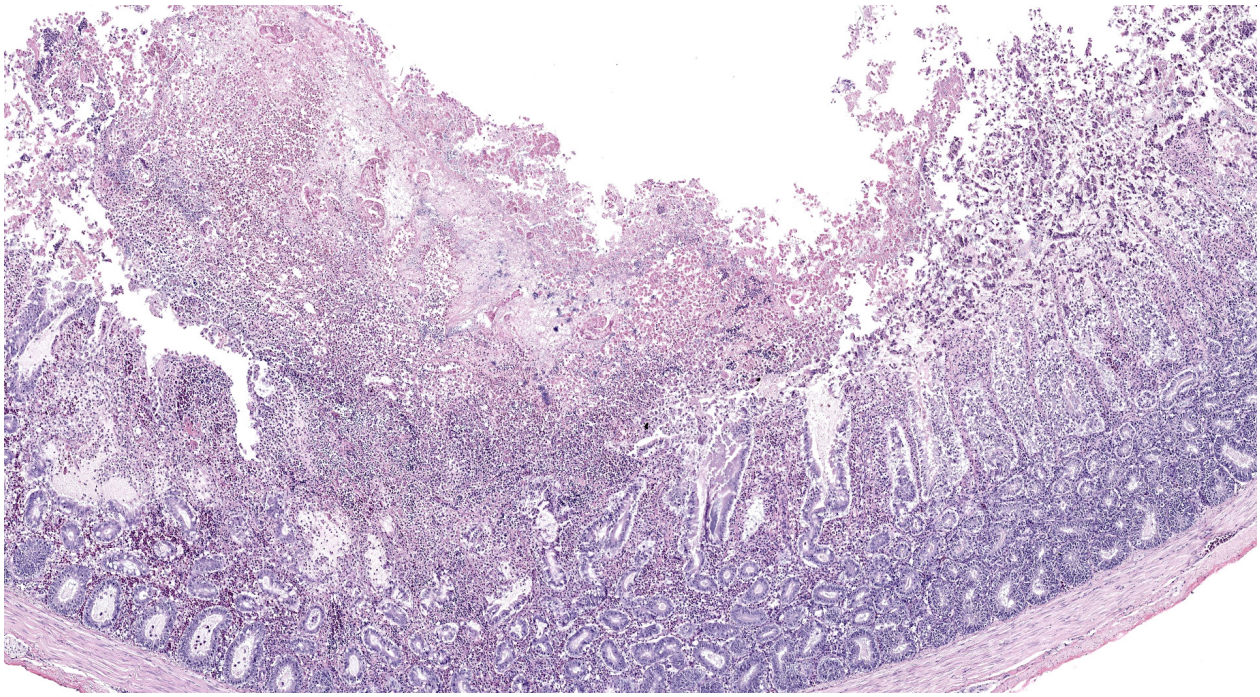

Figure 3-3. Intestine, crow. There is diffuse necrosis of intestinal villi, with a thick covering of fibrin, hemorrhage, and abundant cellular debris overlying the ulcerated mucosa. (HE, 54X)

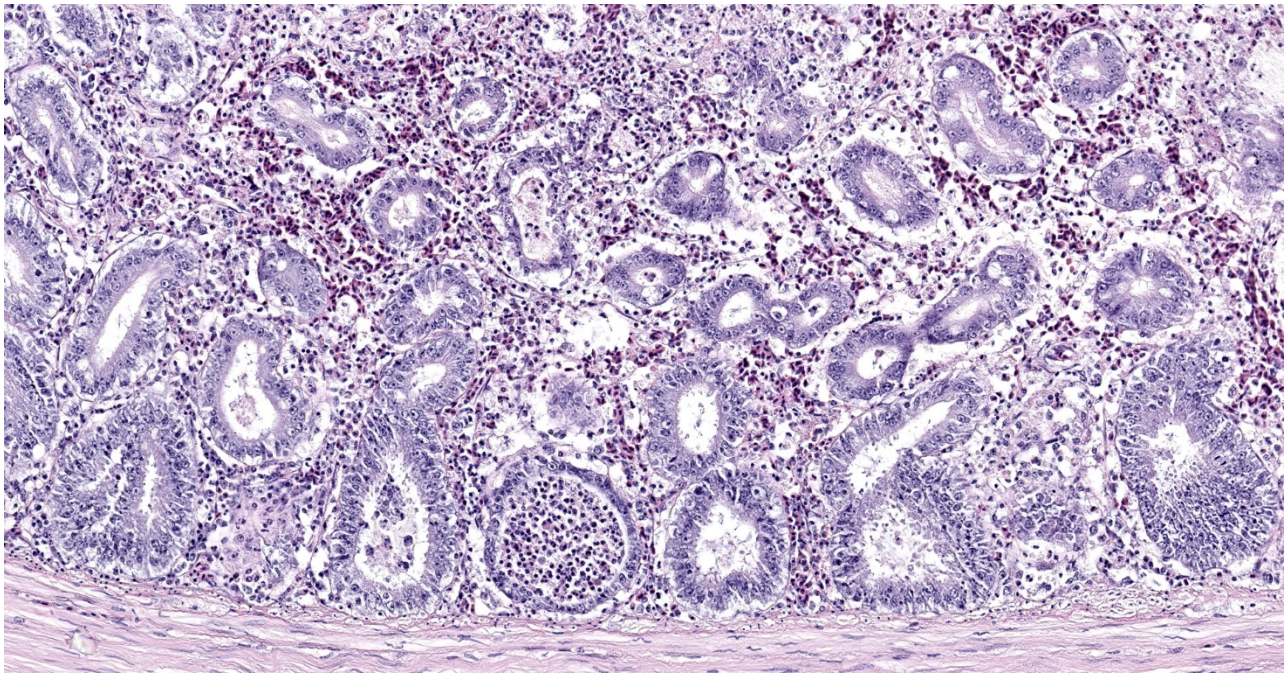

Figure 3-4. Intestine, crow. The lamina propria is markedly expanded by hemorrhage, fibrin, edema, numerous viable and necrotic heterophils, macrophages and lymphocytes. A crypt abscess is at bottom center. (HE, 173X)

viral pathogenicity and induced disease has not been linked to a particular genotype.[4] There have been several case reports of various corvid species with a novel avian orthoreovirus-associated presentation characterized (predominantly) by necrotizing enteritis and splenitis, including a magpie in Great Britain, a hooded crow in Finland, and American crows in North America.[2,5] In the 2019 review by Forzan et al., the authors developed an in situ hybridization probe that was able to demonstrate for the first time direct viral association with the intestinal and splenic lesions in affected crows, strongly supporting causality. Notably, a wide variety of wild birds across multiple orders have also been reported with orthoreovirus-associated disease, including anseriformes, psittacines, and columbiformes, among others. Documentation of these cases is lacking, however the most consistently identified histopathologic lesion in these species is a necrotizing hepatitis.[3]

The examined case provides an example of the lesions of avian orthoreovirus in crows and cautions that orthoreovirus should be considered a differential in sudden outbreaks of high mortality in corvids with evidence of enteritis and splenitis, regardless of season.

Contributing Institution:
San Diego Zoo Wildlife Alliance
Disease Investigations
P.O. Box 120551
San Diego, CA 92112-0551
https://science.sandiegozoo.org/disease-investigations

JPC Diagnosis:
1. Small and large intestine: Enterocolitis, fibrinonecrotizing, hemorrhagic, circumferential, diffuse, severe with crypt and glandular loss.

2. Spleen: Splenitis, necrotizing, multifocal, mild to moderate, with lymphoid depletion.

3. Spleen, macrophages: Hemozoin pigment, moderate.

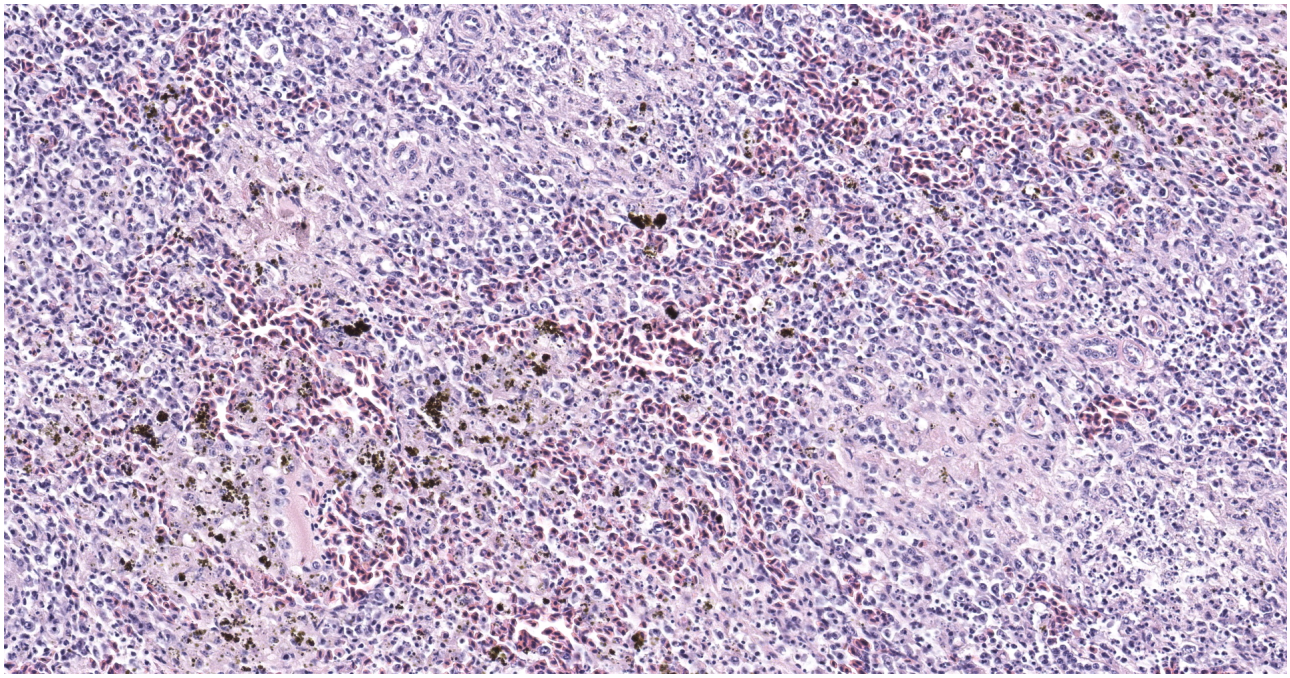

Figure 3-5. Spleen, crow. There is marked splenic lymphoid depletion. There are multiple areas of necrosis (arrows). The dark pigment within macrophages is birefringent (hemozoin). (HE,255X)

4. Adipose tissue, mesenteric: Atrophy, diffuse, marked.

JPC Comment:

The contributor provides an excellent introduction to a viral disease of American corvids colloquially known as "winter mortality of crows". This entity was initially detected through the New York State Wildlife Health Program (NYS WHP) during a period of increased surveillance efforts as a result of West Nile virus. Viral entities such as a rotavirus or paramyxovirus had previously been suggested to be the cause, however, in-situ hybridization (ISH) confirmed an orthoreovirus in 2019. In addition, crows were 32 times more likely develop the typical lesions of necrotizing enteritis, typhlocolitis, and fibrous splenic necrosis when the virus was present.[2]

During a two year period (2016-2017), approximately 20% of crow carcasses examined by the NYS WHP were attributed to reovirosis, which accounted for 70% of all recorded deaths during the winter months, almost exclusively during December and January. Possible explanations for seasonality include immunosuppression due to harsh climate and increased exposure as the result of overcrowding situations during winter roosting.[2]

Orthoreovirus is one of nine genera under the Spinareovirinae subfamily within family Reoviridae. Other notable Reoviridae genera include *Orbivirus* (bluetongue and African horse sickness viruses) and *Rotavirus*.[6] Members of the Reoviridae family are double stranded RNA viruses composed of isometric virons measuring 60-80 nm in diameter with an inner protein coat and one or two distinct icosahedral capsids.[7] All replicate within the host cell cytoplasm, producing characteristic paracrystalline arrays of progeny virions. Virons initially attach to the cell via sialic acid receptors on the cell membrane and subsequently undergo receptor-mediated endocytosis via engulfment and internalization of clathrin-coated pits. These newly formed vacuoles then fuse with lysosomes, resulting in the partial digestion of the

virion's outer shell. Disruption of the outer shell subsequently initiates significant changes in the supramolecular structure of remaining viral components and also activates the transcriptase, resulting in the transcription of mRNA using released subviral particles in the cytoplasm.[7]

The intracytoplasmic, golden-brown, birefringent pigment within splenic macrophages is predominantly negative when stained with Perls' Prussian blue (iron), which is consistent with hemozoin. The moderator discussed the genesis of this pigment and its association with *Plasmodium* and *Haemoproteus* spp. Parasites in the family Plasmodidae consume up to 80% of an erythrocyte's hemoglobin and subsequently produce a toxic pro-oxidant intermediate, α-hematin. The parasite then converts α-hematin to insoluable and inert β-hematin crystals (hemozoin) in a process known as biocrystallization. Similar pigment associated with blood feeding nematodes is also commonly referred to as "hemozoin" although the crystalline structure differs.

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CASE IV:

Signalment:
A young wild mute swan (*Cygnus olor*)

History:
This young mute swan was found dead at the edge of a large pond at a zoological garden. No other history provided.

Gross Pathology:
The bird weighed 1.7 kg and there was almost zero visceral or subcutaneous fat reserves. As this was a young animal (within the first few months of life) neither the pectoral muscles nor feathers were fully developed for flying. The proventriculus was slightly enlarged. On cross-section the proventricular mucosa was thickened and the lumen was narrowed and empty except for a moderate amount of cloudy whitish mucoid fluid. Within the proximal proventriculus, at the esophageal-proventricular junction there were multiple, approximately 1 cm diameter, firm, nodular lesions within the mucosa. These nodules were distributed in a circumferential pattern.

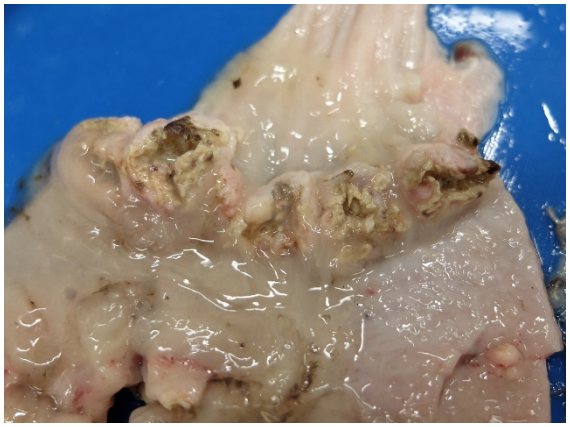

Figure 4-1. Proventriculus, swan. There are numerous nodules at the esophageal-proventricular junction (opened in this photo). (*Photo courtesy of:* Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, http://www.ucd.ie/vetmed/)

There were a few similar lesions in the distal proventriculus. On cut-surface some of these lesions extended transmurally. Others were shallower and affected only the mucosa and submucosa. All the lesions had a central core which was gritty and pale-grey to white.

Laboratory Results:

Aeromonas hydrophila and *A. caviae* were isolated from the lungs.

Eggs recovered from the feces were consistent with *Echinuria uncinata.*

Microscopic Description:

Proventriculus: The mucosa of the proventriculus is disrupted and replaced by large numbers of inflammatory cells which consist mostly of viable and degenerate heterophils, with lesser numbers of lymphocytes and macrophages, as well as cellular debris, fibrin and hemorrhage. There is almost complete loss of mucosal glands. This inflammatory debris extends into and partially occludes the proventricular lumen. Multifocally within the mucosal zone there are numerous sections of parasitic worms (nematodes) and eggs. These worms are surrounded by abundant mixed inflammatory cells, fibrosis, cellular debris, hemorrhage and fibrin. The worms are approximately 250-400µm wide with a 5µm thick cuticle. The cuticle is thickened at regular intervals by many prominent longitudinal ridges. The worms have coelomyarian musculature, a pseudocoelom, and a digestive tract lined by columnar epithelium. The majority of worms within the sections are females and contain myriad eggs. Eggs are oval (sometimes with blunt ends), thick-shelled and most are embryonated. Eggs measure approximately 38µm in length and 18µm in width. Eggs are present both within the nematodes and also scattered extra-corporeally, mixed amongst the inflammatory debris. Underlying the mucosa, the normal boundary between the mucosal and submucosal layers is obscured by inflammation. Multifocally the muscle layers of the proventriculus are markedly disrupted by inflammatory cells (mostly heterophils). The serosa is also disrupted by marked numbers of heterophils.

Other histological findings of note in this bird included marked hepatic amyloidosis and

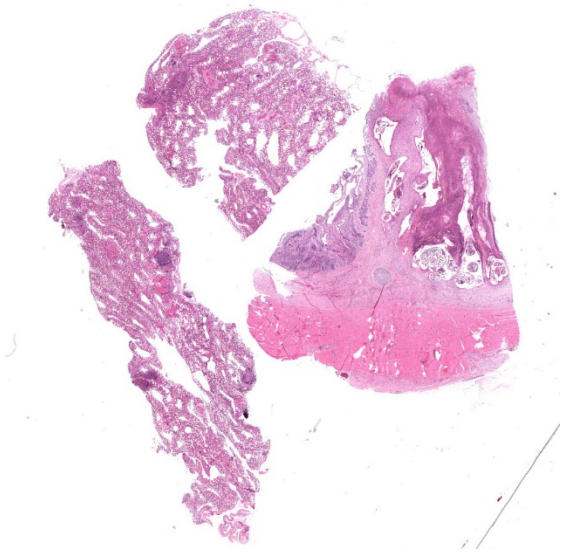

Figure 4-2. Proventriculus, swan. Two sections of lung and a section of proventriculus are submitted for examination. There are numerous cross sections of adult nematodes within the proventricular wall. There are multiple discrete inflammatory nodules scattered throughout the section of lung.

multifocal moderate heterophilic and lymphocytic pneumonia.

Contributor's Morphologic Diagnoses:
Proventriculus: Severe, chronic-active, heterophilic ulcerative and necrotizing proventriculitis, with intra-lesional nematodes and eggs consistent with *Echinuria uncinata*.

Contributor's Comment:
This bird was in poor body condition and it is likely that it died from a combination of factors including debilitation from reduced food intake due to the parasitic lesions in the proventriculus, the effects of liver dysfunction due to hepatic amyloidosis, and pneumonia.

The lesions within the proventriculus are typical of changes seen due to heavy infection with *Echinuria uncinata.* Birds with significant infections are usually weak and in an emaciated condition with a prominent keel.[3] They may have a reduced appetite, and in heavy infections the lesions in the proventriculus may cause reflex retching or choking-like signs if the affected birds attempt to eat. [3]

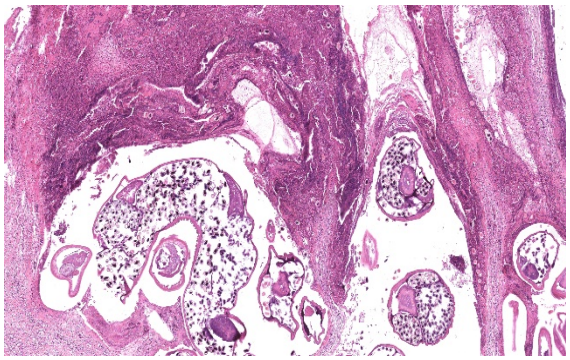

Figure 4-3. Proventriculus, swan: Numerous cross sections of adult nematodes are embedded within abundant necrotic heterophils and cellular debris within the wall of the proventriculus. Nematodes have a cuticle, pseudocoelom, a large intestine with uninucleate cells, and multiple cross sections of uteri laden with embryonated eggs. (HE, 56X)

Birds infected with *E. uncinata* can have a grossly enlarged proventriculus due to worms becoming encapsulated within the mucosal layer.[3,5] The nodules eventually become granulomatous,[5] although for the cygnet in the current case, there was widespread destruction of normal parenchyma and classic granulomas were not seen. In fatal infections, the parasitic nodules and resultant inflammation can cause occlusion of the proventriculus preventing the passage of food.[3] This was the likely outcome in the current case.

E. uncinata is a nematode parasite of numerous species of birds[3,5] and has a wide geographical distribution, being reported from India[3], Europe[3], North America[3], South America[5], Japan[12], Hawaii[11] and Australasia.[4] Many species of waterfowl are affected due to infection with this parasite, although the most significant clinical effects are reportedly seen in anatids (ducks, geese and swans). Approximately 14 species of *Echinuria* are recognized[1] but *E. uncinata* appears to be the species that shows most pathogenicity with attendant clinical effects.[3]

Species of *Echinuria* have a definitive vertebrate host and an intermediate invertebrate host.[3] Definitive hosts for *E. uncinata* are birds, mostly waterfowl, while freshwater crustaceans of the genus *Daphnia* (water-fleas) act as intermediate hosts.[3]

After ingestion of infected species of *Daphnia* by avian hosts, the third-stage larvae of *E. uncinata* are released and disseminate throughout the proventriculus where they further develop to the adult stage. The prepatent period is approximately 30-40 days.[3]

In temperate climates (as was the situation in this case), the numbers of larvae in intermediate hosts reach their highest

numbers in midsummer.[5] In the district where the swan in this case was found, the total rainfall for the previous four months was reduced by 57% compared to the average rainfall during the same months over the previous five years. This resulted in a widespread reduction of water levels in rivers, lakes and ponds. In addition, air temperatures and hours of sunshine were higher for the season in which the swan was growing, compared to the average for the region, which would also have increased evaporation from already depleted water-sources. The effect of this will have been crowding of water sources by waterfowl and concomitant increased fecal contamination and thus increased exposure to water-borne parasites. This has previously been reported for ducks infected by *E. uncinata* on Hawaiian islands[11] and in Canada.[3] It is highly likely that the combination of low rainfall and increased contamination of waterways led to this particular cygnet becoming infected by high numbers of the parasite.

In addition to the proventricular lesions, this bird also had hepatic amyloidosis. Amyloidosis is reported to be a relatively common finding in adult swans[7,8,9,10] and its incidence appears to increase with age.[10] In a study on mute swans in Japan, up to 78% of adult mute swans examined were found to have amyloidosis with the liver being the organ most commonly affected.[10] Causes of amyloidosis in swans include chronic inflammation from 'bumblefoot' or other chronic bacterial infections or parasitic infections.[8,9,10] In contrast to previous studies of swans with amyloidosis, the bird in the current case was quite young. However, it is still reasonable to assume that the accumulation of amyloid was as a consequence of the chronic inflammation in the proventriculus.

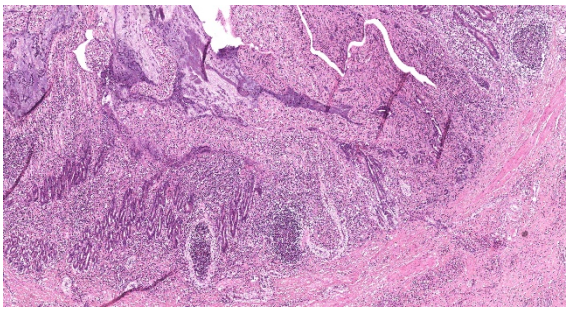

Figure 4-4. Proventriculus, swan. There is marked fibrosis, glandular loss, and chronic inflammation within the adjacent proventricular mucosa. (HE, 77X).

Aeromonas hydrophila and A. caviae were isolated from the lungs of this swan. Both are opportunistic pathogens that concentrate in stagnant waters, and are known to infect water-dwelling animals such as fish and amphibians. Although no bacteria were obvious in the sections of lung examined, the bird did have histological lesions in the lung consistent with a bacterial pneumonia.

Contributing Institution:
Room 012, Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland http://www.ucd.ie/vetmed/

JPC Diagnosis:
1. Proventriculus: Proventriculitis, heterophilic, chronic-active, multifocal, severe with glandular loss and adult male and female spirurid nematodes and eggs.

2. Lung, arterioles, tunica media: Hypertrophy and hyperplasia, circumferential, diffuse, severe.

3. Lung: Pneumonia, heterophilic and histiocytic, multifocal, moderate.

JPC Comment:
Initially described as *Spiroptera uncinatia* by Rudolphi in 1819, this nematode's genus was later transferred to *Echinuria,* following Soloviev's addition of the genus to the family

Acuariidae for a nematode he described in 1912 as *Echinuria jugadornata*, which was likely the same species.[1] Hamann is credited with the discovery of *E. uncinata*'s intermediate crustacean hosts in genus *Daphnia* in 1893, although up to five additional genera have since been shown to support the development of third-stage larvae.[3]

Echinuria are spirurids and belong to the superfamily Acuarioidea, which includes 28 genera, the majority of which are avian parasites. All have characteristically large pseudolabia and cuticular structures located on the anterior aspect known as cordons.[3] Of the approximately 15 species of *Echinuria,* which are predominately reported in waterfowl, *E. uncinata* is the most significant pathogen. Characteristic features of this species, as well as others within its genus, include recurving cordons that extend posteriorally from the anterior aspect, as well as cuticular spines that run along the length of the body. As demonstrated in this case, there is sexual dimorphism with larger females that are nearly twice the length of males, measuring 12-18 mm long compared to 8-10 mm, respectively.[3]

Interestingly, there is interspecies variation amongst waterfowl in regard to susceptibly, with very low susceptibility reported in Northern shovelers (*Ana clypeata*), a species that frequently feeds on *Daphnia* species. The most susceptible species include domestic geese, mallards, gadwalls (*Anas strepera*), Northern pintails (*Anas acuta)*, and common eiders (*Somateria mollissima*). In addition to Northern shovelers, American coots (*Fulica americana*), ruddy ducks (*Oxyura jamaicensis*), and the blue-winged teal (*Anas discors*) are typically resistant.[3]

Other nematodes that may inhabit the proventriculus of birds include other spirurids such as *Tetrameres* spp. and *Streptocara* spp., as well as *Amidostomum*, a tricho-strongyloid. Morphologically, presence of characteristic cuticular spines that extend posteriorly from the anterior end is a unique feature of genus *Echinuria* that allows for differentiation from other spiruids. In addition, *E. uncinata* is most commonly found in the proventriculus whereas species of *Streptocara* and *Amidostomum* are most often found in the ventriculus.[3]

Conference attendees noted multifocal pulmonary heterophilic granulomas addition to diffuse hypertrophy and hyperplasia of smooth muscle cells within the tunica media of arterioles. Although not present within the digital slide used for this conference, schistosome eggs and shell fragments are rarely visible within granulomas in accompanying glass slides submitted with this case. Schistosomes are trematodes that live within the circulatory system and can cause proliferative vascular lesions and endophlebitis.[6] Adults predominantly live within the mesenteric veins or branches and their eggs are disseminated throughout the body. Anseriformes are infected by eight genera, including *Allobilharzia*, *Austrobilharzia*, *Bilharziella*, *Dendritobilharzia*, *Gigantobilharzia*, *Jilinobilharzia*, *Macrobilharzia*, and *Trichobilharzia*.[6]

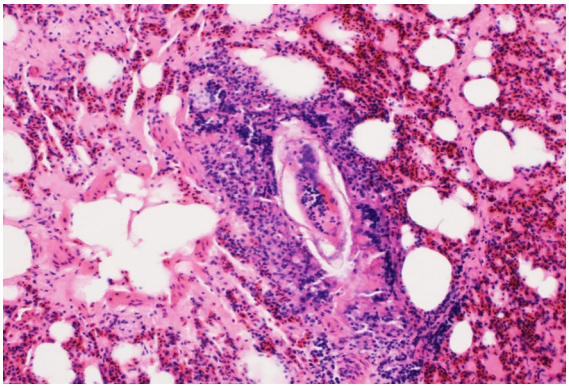

Figure 4-5. Lung, swan: Within the center of heterophilic granulomas, there are cross and tangential sections of schistosome eggs. (HE, 200X)

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