Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 22

6 April 2022

CASE I: RP 18046 (JPC 4153154)

Signalment: Juvenile, male Western Turkey Vulture (*Cathartes aura aura*)

History:
The vulture was found down and unable to rise or fly. Physical exam showed severe paresis and absence of pain sensation in both legs. Due to poor prognosis for return to the wild, humane euthanasia was elected.

Gross Pathology:
Numerous variably sized, well demarcated, white to tan nodules were randomly scattered throughout the kidneys and intestine.

Laboratory Results:
PCR (frozen kidney):

- Avian Poxvirus REV LTR flanking region: positive with 100% identity to *Vultur gryphus* poxvirus genome (GenBank: AY246559)
- Avian Poxvirus core P4b protein gene: positive with 100% identity to Avipoxvirus isolate Hawaii isolate P62 4b core protein gene (GenBank: KC0180210)

Microscopic Description:
Kidney: One section of kidney is examined. Approximately 80% of the renal parenchyma is effaced by a dense inflammatory infiltrate that extends throughout the interstitium, surrounding and replacing tubules and glomeruli. The inflammation is composed predominantly of macrophages with fewer lymphocytes, plasma cells, heterophils, and multinucleated giant cells. Macrophages frequently contain brightly eosinophilic, cytoplasmic material that varies in appearance from unstructured, granular to globular material to discrete, round inclusions with a central clear zone reminiscent of Bollinger bodies. Multifocal areas of necrosis are present throughout the inflammatory infiltrate, characterized by loss of cellular detail and architecture with hypereosinophilic and karyorrhectic cellular debris.

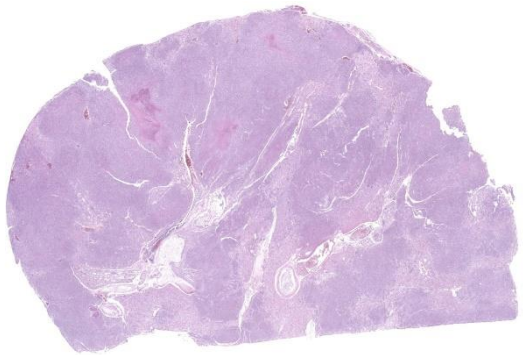

Figure 1-1. Kidney, turkey vulture. Renal architecture is diffusely effaced by a dense infiltrate of inflammatory cells and cell debris. (HE, 5X)

Not included on this slide:
Similar inflammation with intrahistiocytic, intracytoplasmic inclusions were present in the spleen, adrenal glands, thyroid glands, proventriculus, intestine, pancreas, cloaca, and bone marrow. Areas of epithelial hyperplasia with characteristic Bollinger bodies were present in the esophageal mucosa and skin surrounding the vent.

In-situ hybridization for avian poxvirus core P4b protein gene (kidney): diffuse positive labeling within macrophages.

Contributor's Morphologic Diagnoses:
Kidney: marked, subacute, diffuse, granulomatous and necrotizing nephritis with intrahistiocytic viral inclusions (avian poxvirus by PCR and in-situ hybridization)

Contributor's Comment:
Death of this juvenile turkey vulture was due to widespread granulomatous inflammation affecting multiple organs. Macrophages within this inflammation often contained intracytoplasmic inclusions that often resembled typical poxvirus-associated Bollinger bodies. In addition to this inflammatory process within the visceral organs, typical proliferative poxviral lesions were present in the esophagus and skin surrounding the vent. Two PCR assays targeting different regions of the avian poxvirus genome (REV LTR flanking region and core P4b protein gene) detected the presence of avian poxvirus DNA in frozen kidney from this turkey vulture. An in-situ hybridization probe designed to target the avian poxvirus core P4b protein gene also detected the presence of avian poxvirus DNA within infiltrating macrophages in the kidney. Based on the histopathologic and molecular findings, the cause of the widespread inflammatory lesions was attributed to a systemic infection with avian poxvirus. The limb paresis noted clinically in this turkey vulture was due to extension of the inflammation from the kidneys along the sciatic nerves.

Avian poxviruses are DNA viruses in the family *Poxviridae*, subfamily *Chordo-*

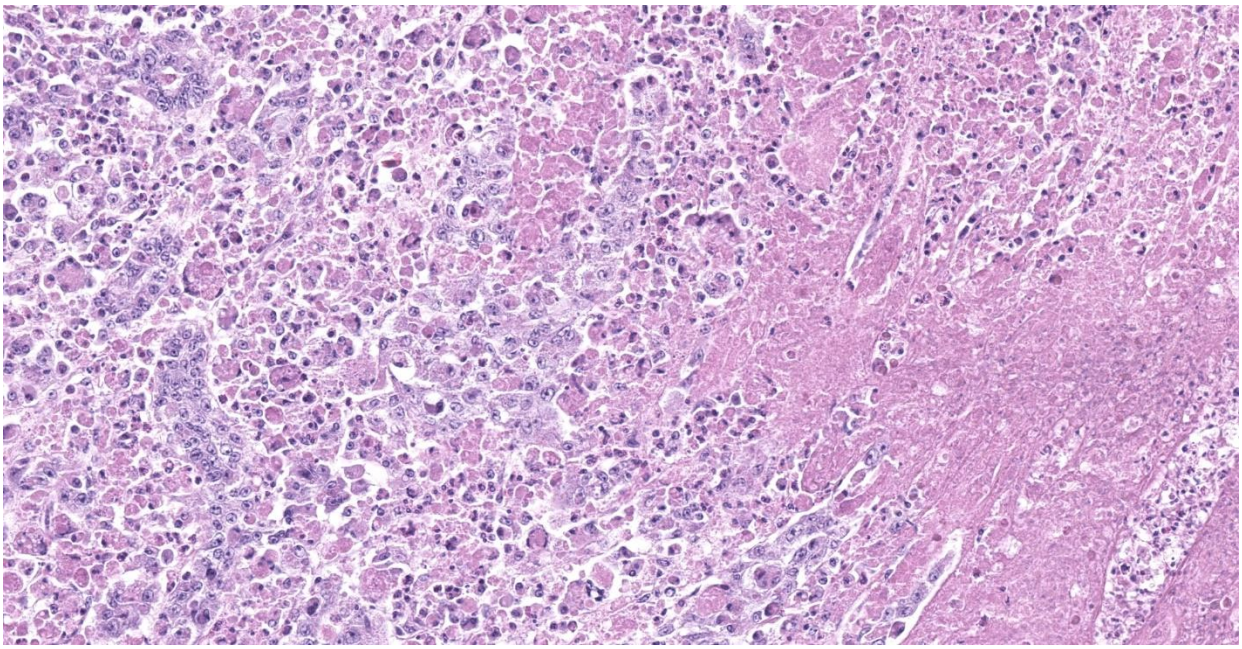

Figure 1-2. Kidney, turkey vulture. There is extensive necrosis of tubules with infiltration by large numbers of debris-laden macrophages, and fewer lymphocytes and heterophils (left) and there are scattered areas of coagulative and lytic necrosis (right). (HE, 190X)

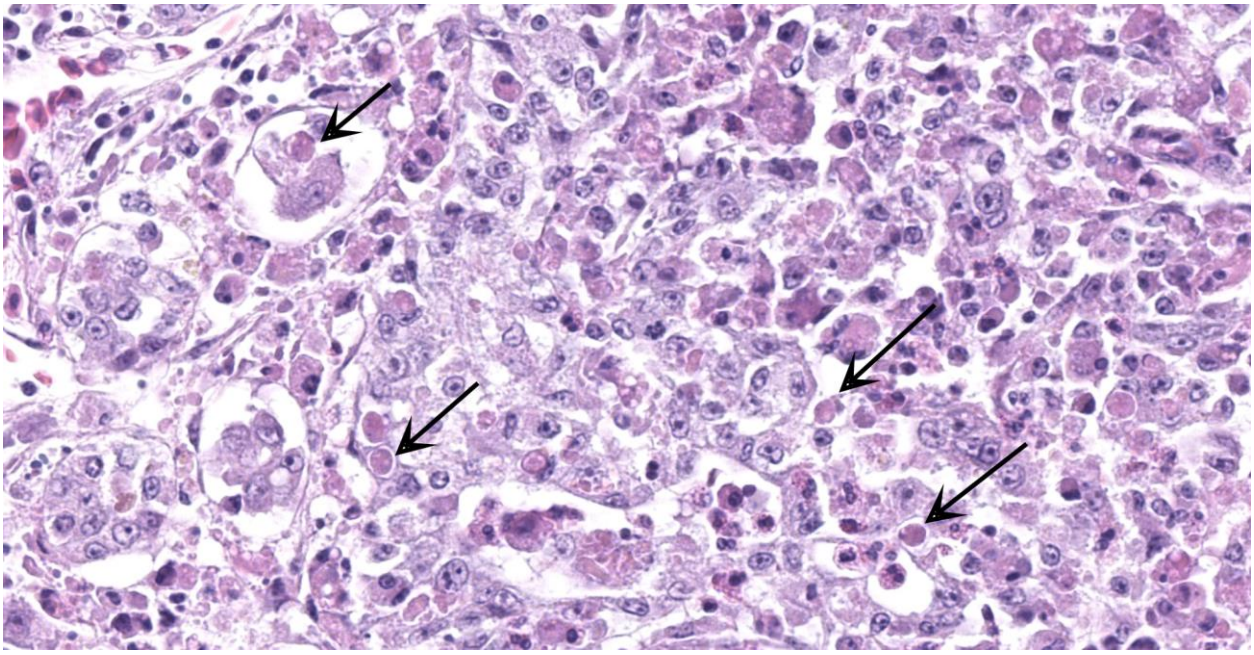

Figure 1-3. Kidney, turkey vulture. Degenerating renal tubular epithelial cells occasionally contain 4um round granular eosinophilic viral inclusions (Bollinger bodies) (arrows). (HE, 250X)

poxviridae, genus *Avipoxvirus* that affect a wide range of bird species

In commercial poultry productions, avian pox is of economic importance due to decreased egg production, stunted growth, and variable mortality rates in infected flocks.[14] Avian poxvirus infections are classically divided into two forms: cutaneous or "dry" pox, and diphtheritic or "wet" pox. Lesions associated with dry pox include one to multiple proliferative, crusty to ulcerated nodules on the non-feathered skin. Birds with wet pox develop proliferative plaques or caseous pseudomembranes on the mucosa of the oropharynx, esophagus, and upper respiratory tract.[14] Both forms of avian poxvirus share similar histologic findings including epithelial hyperplasia and ballooning of epithelial cells with large, cytoplasmic, eosinophilic viral inclusions referred to as Bollinger bodies.[14]

A third form, systemic avian pox, has been rarely reported and most commonly causes a widespread respiratory infection in canaries with high mortality rates.[14] Affected canaries develop fibrinous pneumonia, tracheitis, and air sacculitis with proliferative respiratory epithelial cells containing eosinophilic, intracytoplasmic inclusions.[1,2,8,11,12] Additionally, inclusions were also identified in splenic and thymic reticuloendothelial cells[1], and mononuclear cells in the thymus, bursa of Fabricius, spleen, and bone marrow.[12] Similar avian pox-associated respiratory infections have also been reported in sparrows[2,4] and rosy-faced lovebirds[15], in which inclusions were also identified in coelomic serosal cells[4] and in the bone marrow, bursa of Fabricius, and mononuclear cells within the bones of the digits and skull.[15]

Systemic avian pox can cause predominantly non-respiratory infections as well. Kim et al. 2003 described a captive Andean condor with granulomatous nodules in the heart, lung, liver, kidney, small intestine, pancreas, and spleen. Inclusions were identified within macrophages in these nodules as well as in biliary epithelium, splenic reticulo-endothelial cells, and thymic and bursal epithelial cells. Similar nodular granulomatous

inflammation within parenchymal organs with intrahistiocytic inclusions has been seen in other wild and captive Cathartiformes, including the present case, as well as in multiple species of Passerines, Coraciiformes, and Cuculiformes (Sinnott et al., submitted for publication).

Ten species of avian poxvirus are currently recognized by the International Committee on Taxonomy of Viruses: fowlpox virus, canarypox virus, juncopox virus, mynahpox virus, pigeonpox virus, psittacinepox virus, quailpox virus, sparrowpox virus, starlingpox virus, and turkeypox virus (www.ictvonline.org), although numerous strains exist. Phylogenetic studies using sequences of the core P4b protein gene divides avian poxviruses into three clades: clade A (fowlpox viruses), clade B (canarypox viruses), and clade C (psittacinepox viruses).[6] It is speculated that systemic avian poxvirus disease is associated with infection by strains in the B1 subclade (Sinnott et al., submitted for publication).[1]

The exact pathogenesis of systemic pox in birds is not fully understood, and additional work on host factors, epidemiology of infection, and genomic analysis is ongoing. Systemic involvement of poxviruses is rare in domestic animals with the exception of sheeppox and goatpox, which cause systemic infections often with high mortality in susceptible flocks.[7] Transmission of these viruses occurs via inhalation or skin abrasions, followed by systemic viremia. Affected sheep and goats develop typical poxviral cutaneous lesions in sparsely fleeced areas as well as dermal and subcutaneous edema, vasculitis, proliferative alveolitis and bronchiolitis, and gastrointestinal ulceration. Visceral organs such as the heart, kidney, liver, adrenal gland, thyroid gland, and pancreas are infiltrated by sheeppox cells, mononuclear cells characterized by intracytoplasmic viral inclusions and nuclei with vacuolated chromatin.[7]

Contributing Institution:
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JPC Diagnosis:
Kidney: Nephritis, tubulointerstitial, necrotizing and granulomatous, diffuse, severe, with marked tubular loss and numerous intracytoplasmic viral inclusions (Bollinger bodies).

JPC Comment:
The contributor provides an outstanding review of avipoxviruses and their characteristic manifestations of cutaneous, diphtheritic, and systemic disease in avian species.

Route of transmission plays a significant role toward the development of the diphtheritic and cutaneous forms of the disease. The

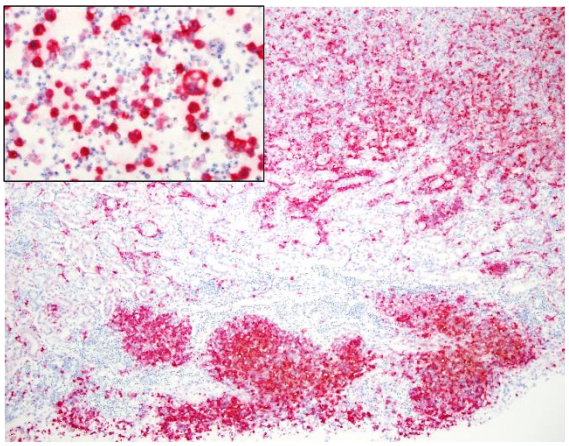

Figure 1-4. Kidney, turkey vulture. There is diffuse positive labeling within macrophages via in-situ hybridization for avian poxvirus core P4b protein. (*Photo courtesy of:* San Diego Zoo Global, Disease Investigations, P.O. Box 120551, San Diego, CA 92112-0551, https://institute.sandiegozoo.org/disease-investigations)

cutaneous or "dry" form of avian pox is commonly transmitted by arthropod vectors but can also be directly transmitted between infected and susceptible birds. Indirect transmission may also occur through contaminated water and food as well as contact with fomites. In contrast, the diphtheritic or "wet" form of avian pox occurs following inhalation of the virus. Although aerosol transmission occurs less commonly than via direct contact, birds housed in close confinement situations such as aviaries or rehabilitation facilities are at increased risk of developing diphtheritic avian pox.[5]

Interestingly, poxviruses indirectly played a major role in the establishment of the United States Department of Agriculture as the regulatory agency for veterinary biologic products. The US Congress passed the Virus-Serum-Toxin (VST) Act in 1914 following an outbreak of foot-and-mouth disease that had been traced to contaminated smallpox vaccine virus that had been imported from Japan. The VST Act authorized the Secretary of Agriculture to prevent the preparation and marketing of worthless, contaminated, dangerous, or harmful virus, serum, toxin, or analogous products. The first license issued for an avian product following passage of the VST Act was provided to the University of California, Berkley on January 13th, 1916 for a fowlpox vaccine labeled "for the prevention of chicken pox."[3]

Mosquitoes, especially *Culex* and *Aedes* spp. are commonly implicated as mechanical vectors of avipoxviruses and are able to retain viable virus on the proboscis for at least 14 days after feeding on an infected bird. The mosquito then mechanically transmits the virus to additional susceptible birds during subsequent blood meals, allowing it to serve as a key bridge between reservoirs and naïve hosts. In addition to mosquitoes, biting midges and mites also been implicated as mechanical vectors of avipoxviruses.[16]

Poxviruses have large complex genomes encoding several genes that interfere with host-cell apoptosis mechanisms. Examples include viral Bcl-2 (vBcl-2) mimics that modulate with the intrinsics1apoptosis pathway as well as other inhibitory strategies targeting the extrinsic pathway, such as TNF receptor homologs. In situations where the intrinsic apoptosis pathway is uninhibited, some viral infections initiate programmed cell death through the activation of BH3 proteins. These sensor proteins promote apoptosis through the neutralization of pro-survival Bcl-2 proteins or by directly facilitating oligmerization of pro-apoptotic proteins (e.g. Bak and Bax) on the mitochondrial outer membrane. Two vBcl-2 mimics specifically sequenced in avipoxviruses include FPV039 from fowl poxvirus (FPV) and CNP058 from canary poxvirus (CNPV). FPV039 suppresses the intrinsic apoptosis cascade through interactions with all pro-apoptotic Bcl-2 proteins, including Bax, resulting in inhibition of mitochondrial pore formation. In contrast, CNP058's interactions are restricted to a specific set of BH3 only proteins (e.g. Bim), resulting in their sequestration and preventing interaction with Bak and Bax, preventing apoptosis.[13]

Participants engaged in spirited discussion in regard to the presence of intracytoplasmic inclusions (i.e. Bollinger bodies) within macrophages. Although participants suspect-ted many cells with intracytoplasmic inclusions to be macrophages, they could not definitively differentiate between macro-phages and sloughed epithelial cells. Participants unanimously agreed cytoplasmic inclusions are present within epithelial cells.

In addition, the moderator utilized the conference as an opportunity to educate participants on Otto von Bollinger (1843-1090), the German pathologist credited with first describing the fowlpox inclusion bodies now bearing his name. Bollinger is also credited with describing the etiologic agent of bovine actinomycosis (*Actinomyces bovis* - "lumpy jaw") in 1877, providing an early description of delayed traumatic intracerebral hematoma in 1891, studies on rabies and hydrophobia, and he was a co-founder and editor of a German journal for veterinary medicine and comparative pathology.

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CASE II: A20-12826 (JPC 4166557)

Signalment:
64-week-old female, Shaver White Leghorn, (*Gallus gallus domesticus*) chicken

History:
Weekly mortality rate increased from 0.42% to 0.50%

Gross Pathology:
The coelomic cavity contained 50 mL serosanguineous fluid with variably sized blood clots. The liver was enlarged, friable, and mottled pale to dark red with yellow foci. Hepatic fractures were associated with hemorrhage into adjacent parenchyma and subcapsular or capsular blood clots. The spleen was diffusely enlarged and friable.

Laboratory Results:
The liver was positive for avian hepatitis E virus nucleic acid by PCR. No pathogenic bacteria were isolated from culture of liver or spleen. Rare *Spirurida* spp. were detected in cecal content by fecal flotation.

Microscopic Description:
In the section of liver, coalescing foci and tracts of lytic necrosis are associated with variable hemorrhage and influx of leukocytes. Necrotic tissue is bordered by edematous fibrous stroma and diffusely infiltrated by lymphocytes, plasma cells, and macrophages with patchy accumulation of homogeneous eosinophilic material. Some portal venules have fibrinoid change with variable infiltration by mononuclear leukocytes. Viable hepatic lobules are disorganized with disrupted plates and individualization of hepatocytes. Spaces of Disse are widened by plasma or fibrin. Portal tracts are infiltrated by lymphocytes and plasma cells. The hepatic capsule is edematous with fibroplasia and hypertrophied mesothelial cells.

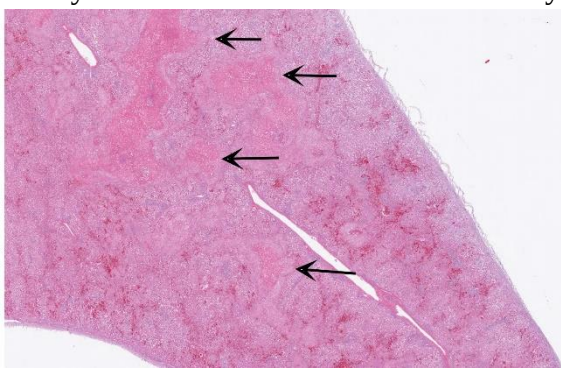

Figure 2-1. Liver, chicken. There is diffuse loss of hepatic architecture and several large areas of necrosis (arrows) at subgross magnification. (HE, 5X)

Contributor's Morphologic Diagnoses:
Multifocal necrotizing and hemorrhagic hepatitis

Contributor's Comment:
Avian hepatitis E virus (HEV) is considered the major cause of hepatitis-splenomegaly syndrome (HS) in chickens, which was first described in Canada in 1991 and subsequently in the US.[1,3] The conditions known as big liver and spleen disease (BLS) in Australia (reported in 1980) and hepatic rupture hemorrhage syndrome (HRHS) in China are attributed to variants of the same virus.[5] The virus, isolated from US cases in 2001, commonly results in subclinical infection, but can also cause slight increases in mortality from 30-72 weeks of age in broiler-breeders and layers as well as decreased egg production.[6] Oral inoculation of 60-week-old specific-pathogen-free chickens with avian HEV resulted in infection and lesions of HS in about one-fourth of the infected chickens.[3]

Gross lesions typically include splenomegaly—though not as consistently as in BLS—along with a large, pale, and friable liver that has multifocal hemorrhages, subcapsular hematomas, and blood clots adherent to the hepatic capsule.[2,3]

Histologically, coalescing foci of hemorrhagic necrosis are associated with lymphocytic hepatitis. Inflammation is most severe around portal venules, described as a lymphocytic periphlebitis; variable number of plasma cells, macrophages, and heterophils accompany the lymphocytes. Segmental lymphocytic portal phlebitis is accompanied by accumulation of homogeneous eosinophilic material that resembles amyloid, but generally is not congophilic.[2,3]

Diagnosis is based on typical history (slight increase in mortality in broiler-breeders or layers), lesions, and—because it is difficult to isolate from cell culture—identification of the virus by avian HEV-specific nested RT-PCR.[5] Transmission is mainly by the fecal-oral route, thus more common in cage-free than in caged chickens.[4] Biosecurity and prevention of fecal-oral transmission is recommended for control because no commercial vaccine is available.[5] Co-infections, e.g., with avian leucosis virus or Marek's disease virus, are common in clinical cases.[5,6]

Avian hepatitis E virus has four major genotypes and is classified in the *Orthohepevirus B* genus along with mammalian hepatitis E viruses.[5] However, although interspecies transmission occurs between chickens and other birds (turkeys experimentally and wild birds), avian hepatitis E viral transmission to pigs, rhesus monkeys, or humans has not been documented, so it is generally not considered to have a public health risk.[5]

Contributing Institution:
Purdue University
Animal Disease Diagnostic Laboratory: http://www.addl.purdue.edu/
Department of Comparative Pathobiology: https://vet.purdue.edu/cpb/

JPC Diagnosis:
Liver: Hepatitis, necrotizing, random, multifocal to coalescing, chronic, severe, with lymphocytic cholangiohepatitis.

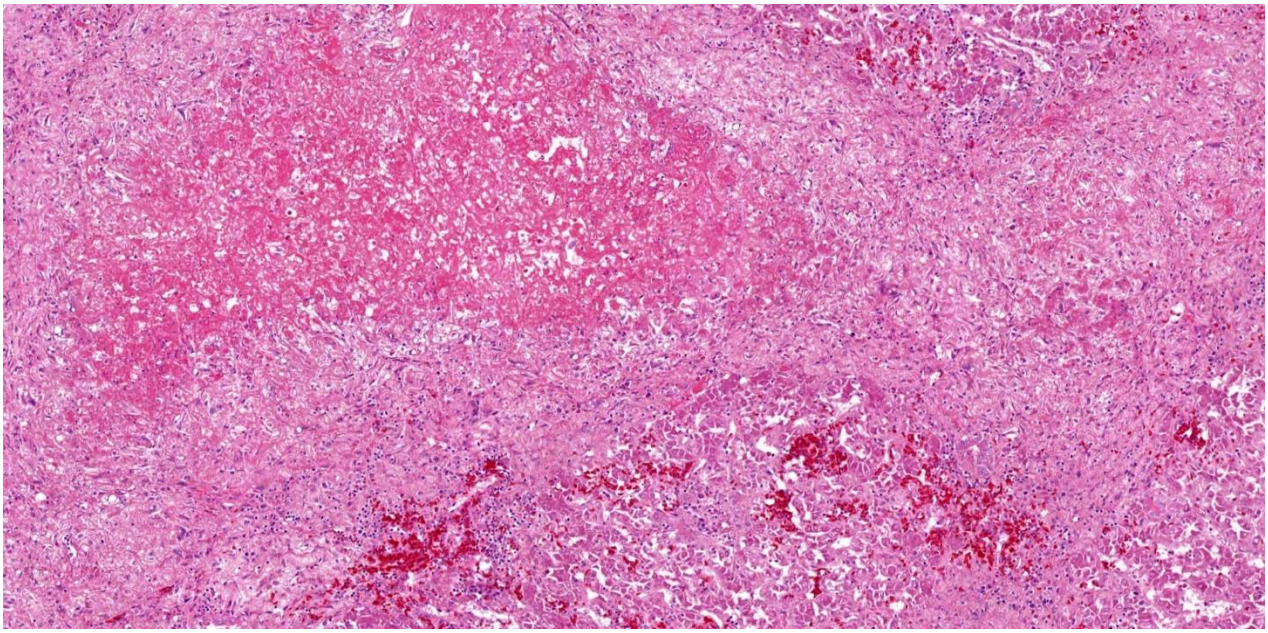

Figure 2-2. Liver, chicken. Bands of coagulative necrosis course across the parenchyma (bottom) and are bounded by granulation tissue. (HE, 131X)

JPC Comment:
The contributor provides a concise summary of Avian hepatitis E virus (HEV), the etiologic agent of big liver and spleen disease, hepatitis-splenomegaly syndrome, and hepatic rupture hemorrhage syndrome in chickens.

Avian HEV is a non-enveloped, single-stranded positive-sense RNA virus composed of an approximately 6.6 kb genome with three open reading frames (ORFs) and non-coding regions at the 5' and 3' ends. The assembly and release of viral particles is mediated by a non-structural polyprotein encoded by ORF1, which is composed of methyltransferase, papain-like protease, viral helicase, and RNA dependent RNA polymerase. ORF2 encodes the capsid proteins, which serve as the major antigentic epitopes associated with immune responses and play multiple roles in both viral replication and pathogenesis. ORF3 encodes a phosphoprotein that associates with the host cell's cytoskeleton.[8]

As noted by the contributor, broiler breeder hens and layers between 30-72 weeks of age are most commonly affected by avian HEV. The disease is typically subclinical with a mortality rate of only 0.3-1.0% in affected flocks. However, these subclinical infections likely contribute toward avian HEV's widespread distribution since the virus is readily shed by infected animals into the environment, resulting in contamination of feed, drinking water, and bedding.[7,8] Following ingestion, the virus undergoes primary replication within the gastro-intestinal tract, with detectable virus within 5 days post infection (dpi) in the colon and cecum under experimental conditions, as well as within the ileum (7dpi), duodenum and jejunum (20dpi), and cecal tonsils (35dpi). Based on mammalian HEV infections, the virus then travels to the liver as a secondary site of replication where it is subsequently released into bile produced by infected hepatocytes. The contaminated bile is then expressed as part of the normal digestive process and eventually excreted into the environment.[7]

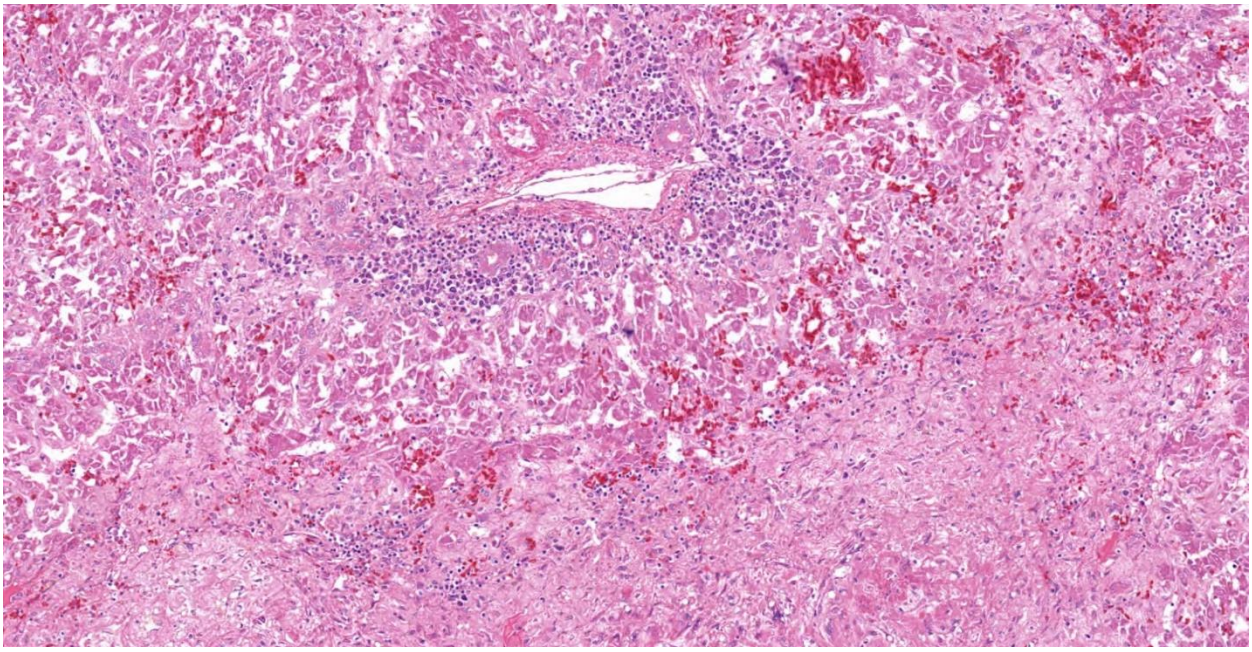

Figure 2-3. Liver, chicken. Bands of coagulative necrosis course across the parenchyma (bottom) and are bounded by granulation tissue. Above, in the viable parenchyma, there are foci of lymphocytic inflammation within portal areas that extend into adjacent hepatic plates, and a portal arteriole has a smudgy eosinophilic wall (fibrinoid necrosis) (arrow). (HE, 181X)

Despite HEV's low mortality rate and typically subclinical infections, it is a disease of economic significance. For example, in Australia nearly 50% of flocks are affected, resulting in an annual loss of eight eggs per hen in affected flocks, leading to the loss of 2.8 million Australian dollars per year. Within the United States, one survey of 1276 chickens from 76 flocks in five states found 71% of flocks to be seropositive. In addition, the study found seropositivity increased based on age, with only 17% of chickens less than 18 weeks of age were seropositive, while the 36% of adult chickens had circulating avian HEV antibodies.[7]

Although the host range of avian HEV is limited to chickens under field conditions, experimental infection has successfully been demonstrated in turkeys (*Melagris gallopavo*), which not only seroconvert but also develop viremia and shed the virus in feces. Attempts to experimentally infect both mice and rhesus macaques have been unsuccessful.[7]

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CASE III: A-808/18 (JPC 4137576)

Signalment:
Muscovy duck (*Cairina moschata*) flock between 36 and 61 days of age.

History:
Ducks from a meat-producing farm in Delta de l'Ebre, Tarragona (Spain) presented with prostration, diarrhea and high mortality rates. They were previously vaccinated against enteric parvovirus. Ten ducks were submitted for necropsy, histopathology and additional diagnostic tests.

Gross Pathology:
Ten birds were sent for necropsy. Feathers and shanks were spotted with feces (soiled

vents). The intestine content was liquid, and the intestinal mucosa showed multifocal petechiae. The intestinal wall was also thickened and multifocally to diffusely reddened, with small amounts of clotted blood.

In some of the birds, liver was enlarged, showing multifocal whitish discolorations. In addition, fibrin deposits were seen over the air sacs and multifocal petechiae in the mucosa of the proventriculus were seen.

In all the birds, cecum scrapings revealed high amounts of flagellated protozoa and helical shaped bacteria.

Laboratory Results:
Cecum samples were taken for microbiologic culture, antibiotic sensitivity test and histopathology. *Escherichia coli* was isolated in all the cases, being the most abundant growth in 7 out of the 10 submitted birds. In 3 cases, *Riemerella anatipestifer* was the only and most abundant isolated bacteria.

In addition, tracheal and cloacal swab samples from all birds were taken to rule out Avian Influenza by means of a conventional RT-PCR. In 3 cases, RT-PCR for *Riemerella*

Figure 3-2. Ceca and ileum, Muscovy duck. There is segmental necrosis and hemorrhage within the cecal and ileal tonsils. (*Photo courtesy of:* Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

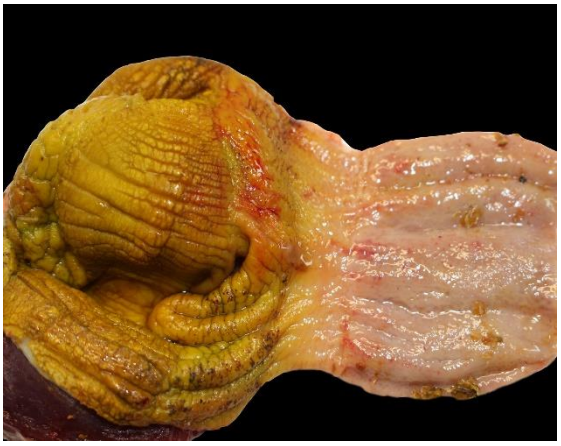

Figure 3-1. Proventriculus, Muscovy duck. There is multifocal petechial hemorrhage within the proventricular mucosa. (*Photo courtesy of:* Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

anatipestifer was also performed from heart and liver samples. RT-PCR for Avian Influenza was negative in all the cases. In contrast, RT-PCR for *Riemerella anatipestifer* was positive in the 3 animals tested.

Microscopic Description:
Cecum: There is diffuse loss of the epithelial lining of the mucosa with an increased number of mitotic figures in the Lieberkühn crypts epithelium. The lamina propria show a marked congestion in its upper part and is markedly expanded by high amounts of both, viable and degenerated, heterophils and lymphocytes. Segmental areas of marked distortion of the whole mucosae (epithelium, lamina propria and muscularis mucosae) are seen. These areas are characterized by high amounts of karyolitic, karyorrhectic debris admixed with heterophils and lymphocytes (lytic necrosis) that extend to the underlying lymphoid tissue of the submucosa. Admixed with this necrotic material as well as in Lieberkühn glands some of the degenerated epithelial cells show marginated chromatin and intranuclear and eosinophilic inclusion bodies. In general, submucosal lymphoid tissue is markedly depleted and high amounts

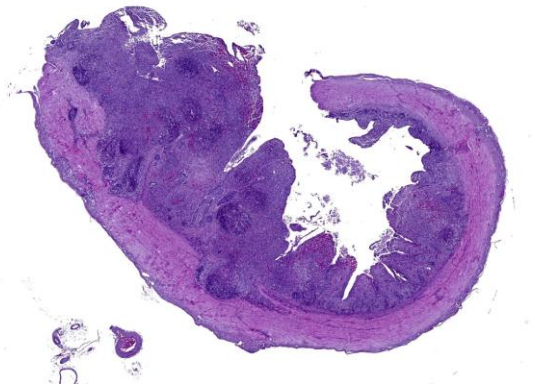

Figure 3-3. Cecum, Muscovy duck. There is diffuse loss, blunting and fusion of mucosal villar projections. Lymphoid tissue is diffusely necrotic with dense aggregates of inflammatory cells viewable at subgross magnification. (HE, 20X)

of pyknotic, karyolytic and karyorrhectic lymphocytes are seen, together with degenerated heterophils.

Sporadically, either free in the intestinal lumen or multifocally attached to the remnants of the epithelial layer or to the denuded lamina propria, moderate to high amounts of bacillary bacterial colonies are observed. Gram staining revealed they were gram-positive bacilli.

Contributor's Morphologic Diagnoses:
Cecum: subacute, segmental to diffuse necrotizing typhlitis with intranuclear viral inclusion bodies in enterocytes and intralesional bacterial colonies.

Contributor's Comment:
The clinical signs together with both gross and microscopic lesions are suggestive of an infection with Anatid herpesvirus 1 (Genus *Mardivirus*, family Herpesviridae and subfamily Alphaherpesvirinae), the causative agent of duck virus enteritis (DVE), also known as duck plague. DVE is an acute, contagious disease of ducks, geese and swans of all ages, characterized by vascular damage, tissue hemorrhages, necrosis and depletion of lymphoid organs, and degenerative changes in parenchymatous organs.[3] It is known to have a global distribution, wherein migratory waterfowl play a crucial role in disease transmission between continents;[1] and it is a cause of significant economic losses in domestic and wild waterfowl due to mortality, condemnations, and decreased egg production.[3]

Natural outbreaks of duck plague have been reported in birds from 7 days of age to adulthood.[1] The incubation period in domestic ducks ranges from 3-7 days. After overt signs appear, death usually follows within 1-5 days.[3] DVE can be transmitted by direct contact between infected and susceptible birds or indirectly by contact with a contaminated environment, mainly the water.[3] In our case, the duckling farm is set in the biggest delta in Catalonia-Delta de l'Ebre-, where many wild birds inhabit.

Secondary bacterial infections with *Pasteurella multocida*, *Riemerella anati-pestifer* and *Escherichia coli* are often seen in natural outbreaks of DVE strains with low virulence in young ducklings as a result of immunosuppressive effect of the virus.[3] In this case, gram-positive bacillary bacteria were histologically seen in 4 out of 10 animals, suggesting a secondary infection with *Clostridium* spp., which can be attributed to a loss of intestinal barrier, dysbiosis and the concurrent immunosup-pression by direct effect of DEV.

DVE rapidly spreads with high mortality rates in high densities farms. Breeder ducks are usually maintained at the same location throughout their productive lives; therefore, once a breeder population is exposed to the virus, DVE infection is self-limiting. In contrast, market ducks are progressively moved, as they mature and are relocated in areas formerly occupied by the next oldest age group.[3] The latter is the scenario seen in our case, as ducks enter the farm at 1-day old

age and are progressively moved to different pens until they go to slaughterhouse.

Regarding latency of the virus, similarly to other herpesviruses, it takes place in the trigeminal ganglion[3], although it has been revealed that lymphoid tissues and peripheral blood lymphocytes (PBL) are as well main latency sites for DEV.[1] Because of the latency phenomenon, carrier states have been described in wild ducks (especially Mallard ducks, which are considered reservoirs of the virus) and also in recovered birds, that can shed the virus periodically. Posterior reactivation has been blamed for precipitating outbreaks in domestic and migrating waterfowl populations triggered by immunosuppression. Recently it has been reported a reactivation of DEV and subsequent outbreaks due to the stresses resulting from the physiological changes in the duration of daylight and onset of breeding, suggesting a certain seasonality in the course of the disease.[1]

DVE virus infects and multiplies in mucosal epithelial cells of the gastrointestinal (GI) tract, macrophages and lymphocytes of lymphoid organs (thymus, bursa of Fabricius, spleen), and later in the liver and endothelial cells (particularly those of small blood vessels, like venules and capillaries). Therefore, gross lesions are associated with disseminated intravascular coagulopathy and necrotic degenerative changes in mucosa and

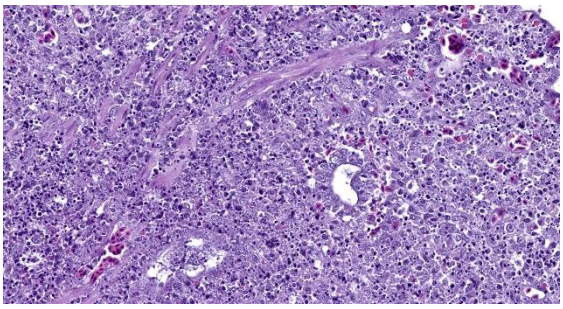

Figure 3-4. Cecum, Muscovy duck: There is diffuse necrosis of cecal tonsillar lymphoid tissue throughout the section. (HE, 380X)

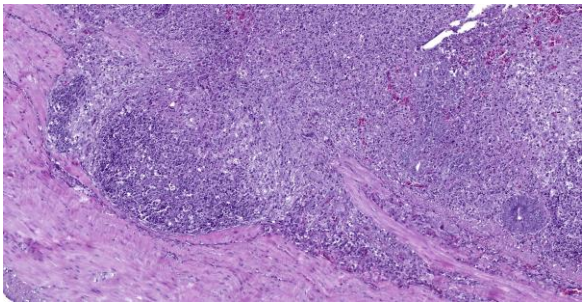

Figure 3-5. Cecum, Muscovy duck. Lymphoid necrosis extends into the underlying submucosal GALT. (HE, 141X)

submucosa of GI tract in lymphoid and parenchymatous organs. Microscopic lesions initially occur in of blood vessels, especially small blood vessels. The endothelial lining is disrupted and connective tissue of the vessel wall become less compact, leading to hemorrhages. Microscopic changes can be found in any visceral organs including those without gross lesions. A hallmark feature of herpesvirus infections is the presence of eosinophilic intranuclear and less frequently cytoplasmic inclusions, seen in chief sites of viral replication.[1,3]

Although a presumptive diagnosis can be made on the basis of gross and histopathologic lesions, isolation and identification of DEV confirm the diagnosis. Differential diagnosis for DEV requires consideration of other diseases producing hemorrhagic and necrotic lesions in anseriformes such as duck virus hepatitis, fowl cholera, necrotic enteritis, coccidiosis, and specific intoxications. Newcastle disease, fowl pox, and fowl plague are reported to produce similar changes in anseriformes; however, these diseases have been infrequently reported.[3]

Samples recommended for virus isolation are liver, spleen, bursa, kidneys, PBL and cloacal swabs. Many techniques have been described to reach a certain diagnosis of DVE (virus isolation, propagation and identification; antigen capture ELISA, different types of PCR or loop-mediated isothermal

amplification – LAMP). The quickest and cheapest tool to use for on-farm and laboratory diagnosis is LAMP.[1,3]

Because there is no specific treatment for infection with DVE, prevention of the disease is highly recommended. It is achieved by maintaining susceptible birds in environments free from exposure to the virus. These measures include quarantine before introducing new animals into a flock and avoiding direct and indirect contact with possibly contaminated material. After DEV has been introduced, control can be achieved by depopulation, removal of birds from the contaminated environments, sanitation, disinfection, and vaccination of all susceptible ducklings.[1,3]

Vaccination has been used as a preventive measure and also for controlling disease outbreaks. Inactivated vaccines have been tried but they have not been as efficacious as modified live virus vaccines. Vaccination guideline is as follows: administration by subcutaneous or IM routes in domestic ducklings more than 2 weeks of age. Moreover, flocks maintained for more than a year are revaccinated annually.[3]

Contributing Institution:
Veterinary Pathology Department,
Veterinary Faculty,
Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.

JPC Diagnosis:
Cecum: Typhlitis, necrotizing, diffuse, severe, with marked lymphocytolysis and intraepithelial intranuclear viral inclusions.

JPC Comment:
The contributor provides a very thorough synopsis of the epidemiology, pathogenesis, and control measures associated with anatid herpes virus 1, the etiologic agent of duck virus enteritis (DVE), also known as "duck plague". Anatid herpes virus 1 presents a major challenge to the commercial duck industry and is associated with both decreased egg production and increased mortality in unvaccinated flocks, despite availability of effective vaccines.[1,2]

Baudet first described features consistent with duck plague in 1926 in the Netherlands, detailing an acute hemorrhagic disease amongst domesticated ducks. Baudet concluded the disease was a duck-adapted strain of fowl plague virus as he was able to induce the disease in domestic ducks by infusing filtered liver suspensions but not in chickens. Anatid herpesvirus 1 was subsequently isolated and identified in 1949, also in the Netherlands.[2] Wild waterfowl were confirmed to be carriers by Friend & Pearson in 1973 following several major outbreaks in domestic and migratory waterfowl. Between 1970-1995, more than 100 outbreaks were reported in regions throughout North America, Europe, the Middle East, and Asia.[1] The disease remains to be a significant issue, as demonstrated by a 2016 outbreak in Egypt that affected approximately 1,400,000 unimmunized ducklings across 10 flocks (6 Muscovy and 4 pekin ducks), with mortality rates ranging from 20-60% on affected farms.[2]

In addition to sudden death, common clinical signs include depression, anorexia, increased thirst, dehydration, weakness, ruffled

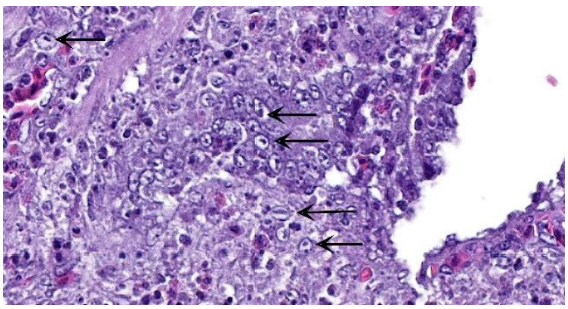

Figure 3-6. Cecum, Muscovy duck. Nuclei of remaining mucosal epithelium contains intranuclear viral inclusions (arrows). (HE, 933X)

feathers, photophobia, head and neck tremors, green watery diarrhea, and hematochezia. In addition, penile prolapse is another clinical sign seen in male birds. Death typically occurs within 5 days of the onset of clinical signs in 60-90%, with higher mortality rates affecting breeders than younger birds, likely due to increased stress.[1]

Interestingly, susceptibility to anatid herpes virus 1 varies within the waterfowl family Anatidae, with some domestic ducks (*Aas platyrhynchos*) such as the white Pekin and Muscovy ducks (*Cairina moschata)* being particularly susceptible. Outbreaks have also been reported in non-Anseriformes water-fowl, including common coots (*Fulica atra*) and crested coots (*Flilica cristata*). In contrast, mallards (*A. platyrhynchos*) are resistant to the lethal effects of the virus and are thought to be a natural reservoir. Additional species that have demonstrated a high prevalence of anatid herpesvirus 1 via loop-mediated isothermal amplification (LAMP) and also may act as carriers include mute swans (*Cygnus olor*), greyleg geese (*A. anser*), tundra bean geese (*A. fabalis*), and grey herons (*Ardea cinerea*).[1] In addition, the virus is resistant to environmental degradation, retaining its ability to infect new hosts for several weeks in unfavorable environments and is able to survive in a pH range of 4-10. Therefore, commercial operations often enact stringent biosecurity measures, including limiting flock access to areas inhabited by waterfowl, chlorinating water, disinfecting fomites (e.g. footwear), promptly removing carcasses, and quarantining new arrivals, in addition to vaccination programs.[1]

Ducklings of vaccinated and/or previously challenged breeders possess short lived immunity to anatid herpesvirus 1, which can interfere with vaccination efficacy. One study concluded the optimal time for primary vaccination to be at 35 days of age, with a booster 5 months after the initial inoculation.[1]

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CASE IV: 629-183 (JPC 4136414)

Signalment:
Adult musk lorikeet (*Glossopsitta concinna)*

History:
Wild bird found dead and submitted for necropsy examination.

Gross Pathology:
There were multiple white to beige dermal exophytic nodules with papillary surfaces arising from the skin in various locations on the body. The largest mass (40 x 30 x 20mm in size) was under the left wing, and smaller masses were present bilaterally on the right radius and ulna, and on the patagial skin. Further masses arose from the ventral neck (10 x 5 mm), the dorsal head (2 x 1mm), and adjacent to the cloaca (20 x 10 x 5 mm). The bird was in thin body condition, but no other findings were noted on gross examination.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Feathered skin, dermis and subcutis: A multilocular cystic structure is expanding within the dermis and compressing adjacent structures, interspersed and supported by septa of fibrous connective tissue. The cysts are lined by thin stratified squamous epithelium, and the lumina of the structures are filled with abundant keratin. Embedded within the keratin, there are large number of arthropod cross sections characterized by a fragmented chitinous exoskeleton, partially intact, jointed appendages, a body cavity with striated muscle, multiple cross sections of gastrointestinal tract, and male and female reproductive tracts. Multifocally the lining of the cysts is eroded, and the surface is overlayed by a deposit of fibrin containing abundant degenerate heterophils. Occasional aggregates of cocci, bacilli, and yeasts are also present within some of the cystic structures.

Contributor's Morphologic Diagnoses:
Feathered skin, dermis and subcutis: Feather follicle cysts, multifocal, severe, chronic, with intralesional mites and mixed bacteria and yeasts.

Figure 4-1. Presentation, musk lorikeet. Exophytic alopecic dermal nodules cover large areas of the bird's skin. (*Photo courtesy of:* Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Werribee, Victoria, Australia http://fvas.unimelb.edu.au)

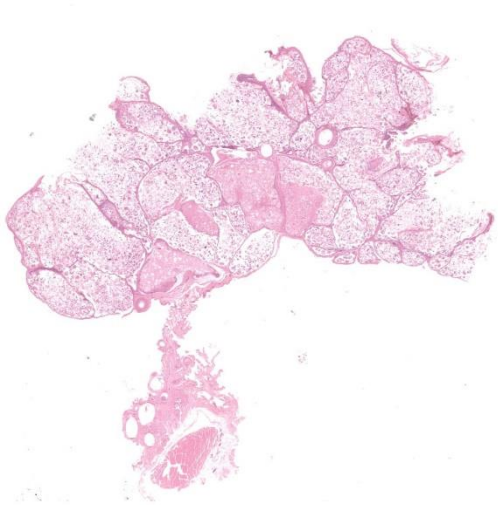

Figure 4-2. Feathered skin, musk lorikeet. At subgross magnification, there are numerous dilated feather follicles containing mites. (HE, 4X)

Contributor's Comment:
The gross and histologic presentation in this case is compatible with feather follicle cysts caused by feather mites. Grossly, feather follicle cysts usually present as a bulge at the base of the feather, or as an oval or elongated dermal nodule or mass filled with yellow-whitish materials. They are most commonly found on the wings and over the back, but can appear anywhere on the integument. Such cysts can occur as a heritable condition of uncertain pathogenesis, especially in soft-feathered canaries, such as Gloucester and Norwich breeds,[11] but the condition has also been reported in macaws, mynahs, and Amazon parrots.[8] Feather cysts have also been found in parakeets and parrots as an acquired condition secondary to infection, trauma or any other condition that interferes with the feather growth.[9]

In this case, the formation of these cystic structure is caused by the accumulation of numerous arthropod mites within the feather follicle. The follicular epidermis of infested

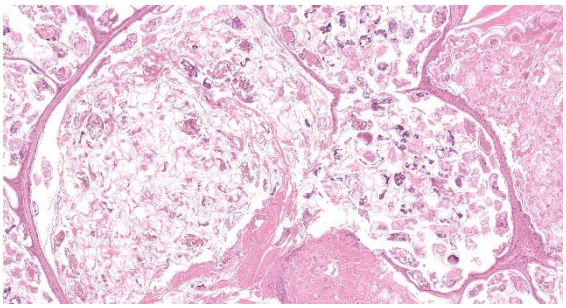

Figure 4-3. Feathered skin, musk lorikeet. Higher magnification of the contents of a dilated follicle with numerous cross sections of mites and abundant keratin debris. (HE, 48X)

follicles proliferates to form a cystic structure which is distended with mites. Mites extracted from the follicle cysts in this case were identified as *Harpyrhynchus rosellacinus* according to the taxonomic keys of the prostigmatic mites (the location of Setae vi and ve; the characters of cuticle; and the segmentation of leg IV).[2,7] The Harpyrhynchidae family is exclusive to birds,[1] and species that have been discovered in Australia include *H. kakatoe*, *H. monstrosus*, and *H. rosellacinus.*[2] Only *H. rosellacinus* is known to cause nodular lesions of the feather follicles in lorikeets, and it is the only species of mite known to cause feather cyst formation in birds.[4,6] The mite has been found in the feather follicles of musk lorikeets (*Glossopsitta concinna*), eastern rosellas (*Platycercus eximius*), scaly-breasted lorikeets (*Trichoglossus chlorolepidotus*), and rainbow lorikeets (*Trichoglossus moluccanus*).[2]

Several species of feather mites are relatively common in birds, including blood sucking mites, such as *Dermanyssus* spp. and *Ornithonyssus* spp., which may cause anaemia and death in heavy infested cases;[6] and non-pathogenic mites, such as *Protolichus* spp. and *Dubininia* spp., which feed on feather and skin debris only.[5] Unfortunately, most types of mites cannot be by identified in histological section due to the lack of distinguishing morphologic characteristics.

Bacteria and yeasts (most consistent with *Malassezia* sp.) presents with mites in cysts are most likely an incidental finding in this case.

Contributing Institution:
Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Werribee, Victoria, Australia
http://fvas.unimelb.edu.au

JPC Diagnosis:
Feathered skin: Follicular ectasia, regionally extensive, marked, with innumerable mites, and ulcerative dermatitis.

JPC Comment:
Feather follicle cysts occur when a developing feather fails to breach the epidermis, resulting in the formation of a cyst as the feather continues to grow within the follicle. Therefore, any pathologic condition interfering with feather growth and/or emergence such as feather follicle dysplasia, endocrinopathy, neoplasia, malnutrition,

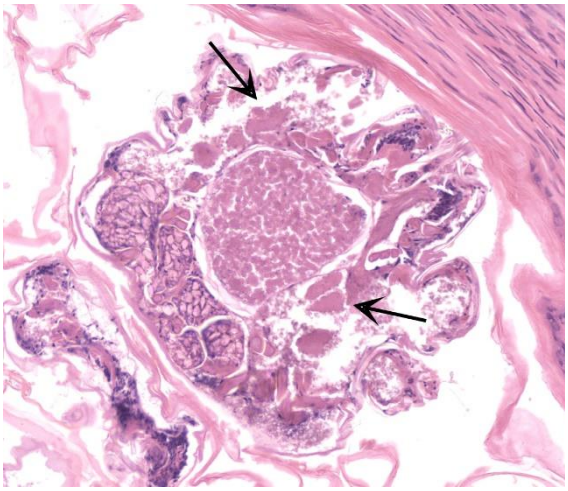

Figure 4-4. Feathered skin, musk lorikeet. High magnification of a cross section of a follicular mite with jointed appendages and underlying skeletal muscle (arrows). (HE, 380X)

superficial injury (e.g. feather picking), infestation, infection, or a combination of these factors predispose the bird to forming feather follicle cysts. Young birds are commonly affected by these benign lesions as their feathers initially emerge whereas older birds are more commonly affected as new feathers attempt to emerge during moulting, with the risk feather cyst development increasing with each subsequent moult.[10]

Also known as 'hypopteronosis cystica', 'pterylofolliculosis cystica', and pluma-folliculomas, feather follicle cysts are relatively rare in domestic fowl species, while they're more common in small wild and caged birds. Feather follicle cysts exhibit a range of gross features and may be wet or dry, solitary or multiple, firm or soft, open or closed, fixated or sessile thin walled nodules measuring up to 4cm. When exposed, the luminal contents are typically composed of soft friable concentric lamellar structures centered on a malformed feather.[10]

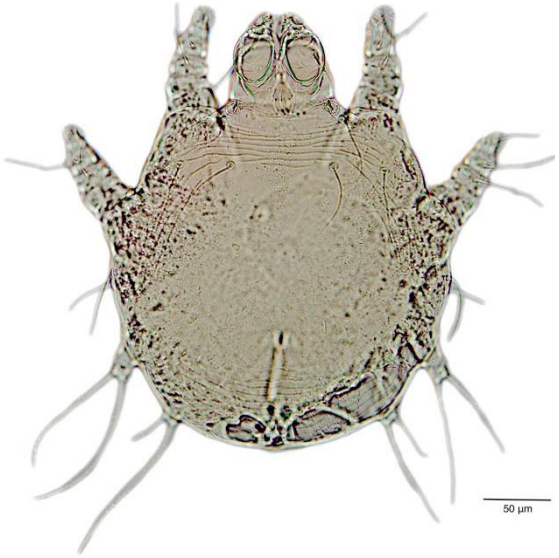

Figure 4-5. Whole mount preparation of *Harpyrhynchus rosellacinus.* (*Photo courtesy of:* Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Werribee, Victoria, Australia http://fvas.unimelb.edu.au)

Commonly known as 'quill mites', mites within order Prostigmata (including *Harpyrhynchus* spp.) and the superfamily Analgoidea (order Astigmata) infest multiple avian species, including gallinaceous birds, passerines, pigeons, ratites, parrots, and other psittacines. In some cases these mites are host specific whereas others infest multiple species. In contrast to other mites that feed on blood or sebaceous fluid, quill mites feed on the smooth, hollow, colourless part of the feather that does not have any barbs and inserts into the skin (i.e. the quill or calamus); they do not invade the rachis, a continuation of the quill to which the barbs attach, forming the vane. Clinical signs of quill mite infestation are typically mild and include irritation and pruritus, feather picking, and feather loss. With the aid of magnification, observation of mites within powdery quill material of malformed or broken feathers is typically diagnostic.[3]

During conference discussion, there was debate in how to best represent the primary process taking place in the tissue. A minority of participants favoured heterophilic folliculitis, whereas a majority favored follicular ectasia as being the cause of the heterophilic folliculitis given the previously discussed pathogenesis associated with the formation of feather follicle cysts.

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