Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 9

30 October 2019

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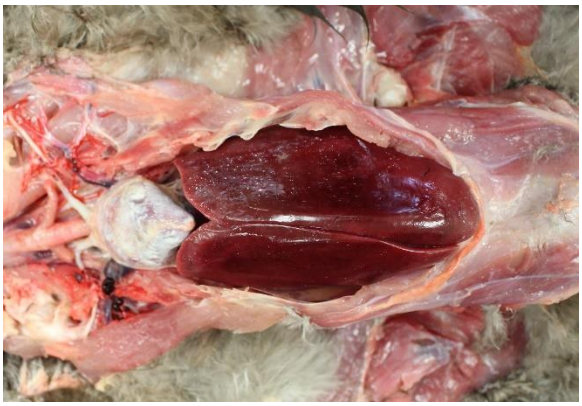
CASE I: WSC18-19 #1 (JPC 4117005).

Signalment: Three-year-old Peahen (*Pavo cristatus*)

History: A reportedly three-year-old peahen was presented deceased for necropsy examination following a weeklong history of being lethargic with no mobility in her legs. She was eating and drinking well. The peahen had whitish diarrhea few days before death. The other 11 birds in the group were acting normally.

Gross Pathology: The bird was in good body condition with adequate pectoral muscling. Diffusely, the pericardial sac was mildly thickened by a large amount of white chalk-like material which was adhered to its surface. The kidneys appeared pale and on cut section, there was white granular material throughout the renal parenchyma.

The inner wall of the coelomic cavity and the surfaces of the visceral organs including the liver and abdominal air sacs were covered in small quantities of fine white powder-like material. The duodenal contents were mixed with white granular material throughout.



Liver, heart, peahen. Diffuse thickening of pericardium and pinpoint lesions on liver consistent with urate deposition. (Photo courtesy of: Kansas State University Veterinary Diagnostic Laboratory/Dept. of Diagnostic Medicine/Pathobiology, http://www.ksvdl.org)

The right stifle joint had significant sub-capsular hemorrhage that extended into the adjacent muscle. Subcutaneously and intramuscularly along the medial aspect of the left tibiotarsus were extensive dark red areas (hemorrhage).

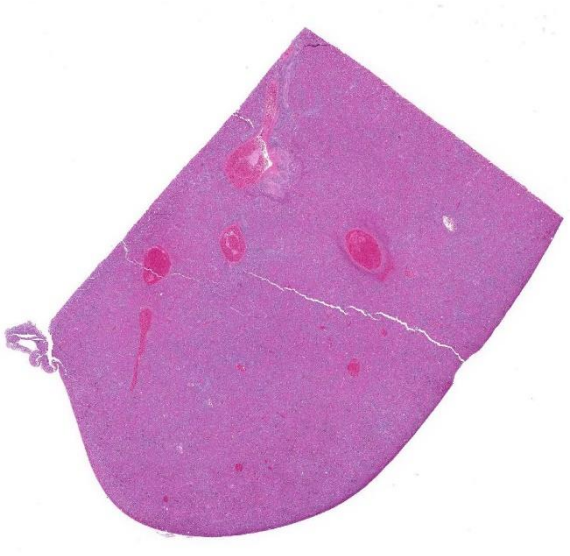
No significant gross lesions were seen in the oral cavity, trachea, lungs, esophagus, crop, proventriculus, gizzard, jejunum, caecum, colon, uterus, ovaries, liver, lymph nodes, spleen or brain.

Laboratory results: The pooled liver and kidney sample was positive for Marek's disease virus by real-time PCR.

Microscopic Description: Liver: Diffusely, the normal hepatocellular architecture is disrupted by an accumulation of large numbers of medium to large sized lymphocytes and lymphoblasts that moderately expand the sinusoidal spaces. The pleomorphic lymphoid cells are more concentrated around the central veins (centrilobular area). These lymphoid cells contain small to moderate amounts of basophilic to amphophilic, homogenous cytoplasm with round to oval nuclei with coarsely stippled chromatin and indistinct nucleoli. Approximately 20-25% of the neoplastic lymphocytes appear degenerate with fragmented nuclei that appear as basophilic pyknotic bodies of variable sizes. Similar lymphoid infiltrates were present in the kidneys, proventriculus, ventriculus, small intestine, ceca, spleen, ovary, and globes.

Immunohistochemistry:
Immunohistochemical staining showed strong positive reactivity of the lymphocytes for CD3 and negative for CD20 and CD79a.

Contributor's Morphologic Diagnosis:
Liver: Lymphoid infiltration, diffuse, severe, with scattered hepatocyte necrosis.



Liver, peahen. A section of liver is submitted for examination. Even at low magnification, a retiform pattern of cellular infiltration, especially prominent in proximity to blood vessels is evident. (HE, 7X)

There are approximately 3-5 mitotic figures per HPF (400X). Multifocally, the hepatocytes are mildly compressed by the infiltrating lymphoid cells and occasionally few hepatocytes show single cell necrosis with hypereosinophilic cytoplasm, pyknotic nuclei, and a clear space around them.
Contributor's Comment: Liver: Diffusely, the normal hepatocellular architecture is disrupted by an accumulation of large numbers of medium to large sized lymphocytes and lymphoblasts that moderately expand the sinusoidal spaces. The gross lesions of visceral gout with deposition of urates on the heart, liver, and kidneys were most likely the result of impaired renal function caused by infiltration of neoplastic lymphocytes. Many neoplastic diseases in poultry are caused by viral etiologies. A list of the viral induced neoplastic diseases seen in poultry are provided in Table 1.

Table 1: Transmissible neoplasms [2].			
Virus type	Nucleic acid type	Virus classification of etiological agent	Neoplastic diseases
Retrovirus	RNA	Leukosis/sarcoma Group	**Leukoses** Lymphoid leucosis Erythroblastosis Myeloloblastosis **Sarcomas and other connective tissue tumors** Fibrosarcoma, fibroma Myxosarcoma, myxoma Osteogenic sarcoma, osteoma Histiocytic sarcoma Related neoplasms Hemangioma Nephroblastoma Hepatocarcinoma Osteopetrosis
		Reticuloendotheliosis Group (REV)	Reticuloendotheliosis Lymphoid leucosis
Herpesvirus	DNA	Marek's disease virus	Marek's disease

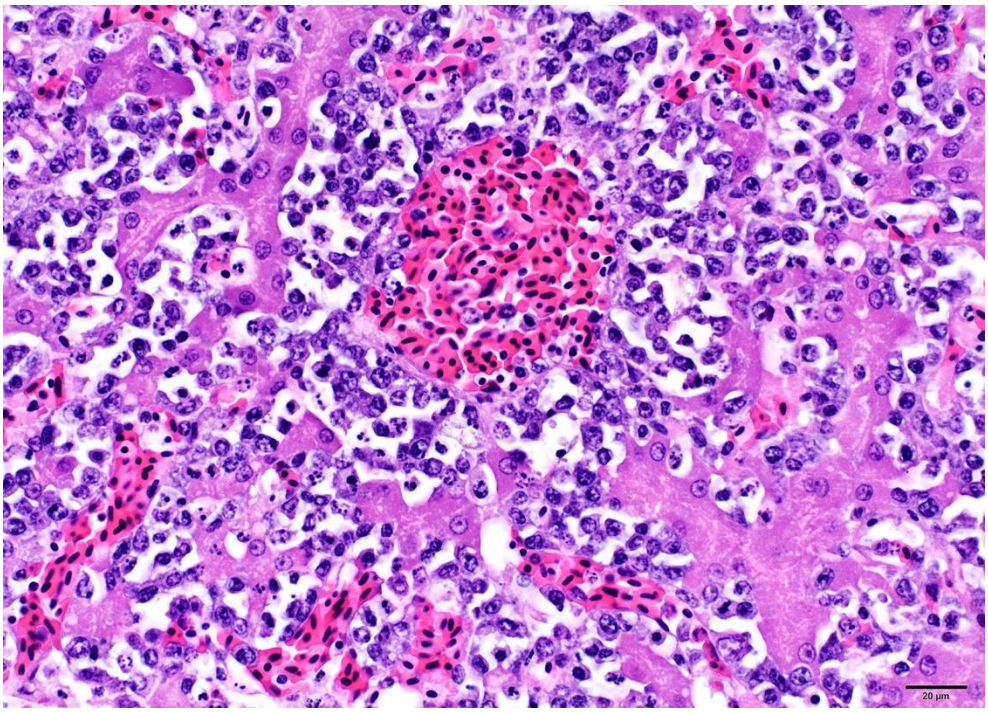
(Table adapted from Swayne DE, ed., *Diseases of Poultry* 2017.)

Confirmatory diagnosis of the visceral form of Marek's disease requires histopathological examination, identification of MDV, by PCR and/or demonstration of MATSA antigen on tumor cells by immunohistochemistry.

Marek's disease is a cell-associated lymphoproliferative disease that is commonly seen in domestic chickens and less commonly in other birds. This is believed to be the first report of Marek's disease in this species of peafowl. A novel herpesvirus that was most closely related to gallid herpesvirus-3 was previously reported in three peafowls that had hepatocellular necrosis with intranuclear eosinophilic inclusions.[9]

The causative agent of Marek's disease, gallid herpesvirus 2, belongs to the genus *Mardivirus*, subfamily: Alphaherpesvirinae and family: Herpesviridae. The genus *Mardivirus* consists of different serotypes: Serotype 1 MDV (gallid herpesvirus type 2), Serotype 2 MDV (gallid herpesvirus type 3) and Serotype 3 herpesvirus of turkeys (meleagrid herpesvirus type 1). Serotypes 2 and 3 are non-oncogenic.[7] The serotype-1 MDV consists of Marek's disease virus of varying pathogenicity based on which they are classified as mild (mMDV), virulent (vMDV), very virulent (vvMDV) and very virulent + (vv+MDV).[8] Clinical signs can occur in chickens as early as 4 weeks of age and are commonly seen in birds between 12 and 24 weeks of age. The disease occurs in four distinct syndromic forms:[7]

1) Classical form: Mainly characterized by neurological signs with partial or complete



Liver, peahen. Neoplastic lymphocytes fill hepatic sinusoids around a central vein with mitotic figures and evidence of cellular necrosis. Several mitotic figures and numerous apoptotic cells are present within the neoplastic population. Neoplastic cells are also present within the lumen of the central vein. (H&E, 400X) (Photo courtesy of: Kansas State University Veterinary Diagnostic Laboratory/Dept. of Diagnostic Medicine/Pathobiology, http://www.ksvdl.org)

paralysis of legs and wings. Other common clinical signs include torticollis, dilation of crop, gasping, and respiratory distress depending on the nerves affected.

2) Acute form: Involves the formation of lymphomas in the visceral organs causing anorexia, depression, weight loss, and diarrhea.

3) Acute cytolytic form: Commonly seen in infections caused by vvMDV strains. It is characterized by high mortality with severe atrophy of lymphoid organs.

4) Transient paralysis: This is an uncommon form that lasts for about 24-48h and is usually associated with edema of the brain causing varying degrees of ataxia, paresis or paralysis of the legs, wings and neck.

Following infection by direct or indirect aerosol route through inhalation of cell-free virus particles within feather dander, the pathogenesis of MDV is very complex and is influenced by the age, immune status, and genetic susceptibility. MD pathogenesis is characterized by four phases:[3,10]

1) Early cytolytic phase: seen within 2-7 days post infection (dpi). Initial infection of lung epithelial cells and production of viral Interleukin-8 (vIL-8) recruits the innate immune cells resulting in infection of macrophages and B-cells. By as early as 24 hr pi, the macrophages and dendritic cells can disseminate the virus from lungs to B cells and CD4+ T cells in the bursa of Fabricius, spleen, and thymus. During the cytolytic phase, large numbers of B-cells within the spleen along with CD4+ T cells in cecal tonsils undergo apoptosis and contribute to immunosuppression. A semi productive lytic viral replication in B cells and production of vIL-8 leads to recruitment and infection of T-cells which then leads to viremia and systemic spread of infection.

2) Immune evasion and latency phase: occurs between 7-10 dpi. MDV integrates into the genome of the infected CD4+ T cells leading to immune evasion and establishment of latency.

3) Cutaneous infection, replication and shedding: latently infected CD4+ T cells migrate to cutaneous feather follicles, where

they infect the feather follicle epithelium. The resulting fully productive viral replication causes syncytia formation, and secretion of mature virions in skin dander. Aggregates of small lymphocytes with intranuclear inclusions can be seen in perifollicular areas by 7 dpi. These lymphoid aggregates develop into either cutaneous tumors or degenerate to form necrotic foci.

4) Proliferative phase: occurs around 28 dpi and is characterized by formation of CD4+ T cell visceral lymphoma. Meq (Marek's EcoQ) gene has been shown to be important for transformation and Meq protein is consistently expressed in lymphoma cells and tumor cells[5]. The infection in transformed cells is nonproductive.

The two important and most common lymphomatotic diseases are Marek's disease and lymphoid leukosis (ALV) differential diagnosis of the two is confusing. Table 2 describes the important features for their differential diagnosis.

Table 2: Gross and microscopic features for differential diagnosis of Marek's disease and lymphoid leukosis[1]

Feature	**Marek's disease**	**Lymphoid leukosis**
Age	Few days to many weeks	Not less than 16 weeks
Clinical signs	Frequent paralysis	Nonspecific
Incidence	Usually more than 5% in unvaccinated flocks	Rarely more than 5% of infected flocks
Gross lesions		
Neural enlargement	Frequent	Absent
Bursa of Fabricius	Diffuse enlargement or atrophy	Nodular tumors
Proventriculus, skin and muscle tumors	May be present	Usually absent
Microscopic lesions		
Neural involvement	Frequent	Absent
Cytology of tumors	Usually pleomorphic lymphoid cells consisting of lymphoblasts, small, medium and large lymphocytes and reticulum cells	Lymphoblasts of uniform morphology, usually of clonal origin
Category of neoplastic lymphoid cell involved	T lymphocyte	B lymphocyte

(Table adapted from Pattinson M et al., *Poultry Diseases* 2008.)

Marek's disease virus is transmitted horizontally only, and appropriate hygiene precautions and vaccination can prevent its spread in hatching eggs and day-old chicks. ALV and REV can be transmitted both horizontally and vertically and avian leukosis and REV can be controlled by virus eradication at the primary breeding level.

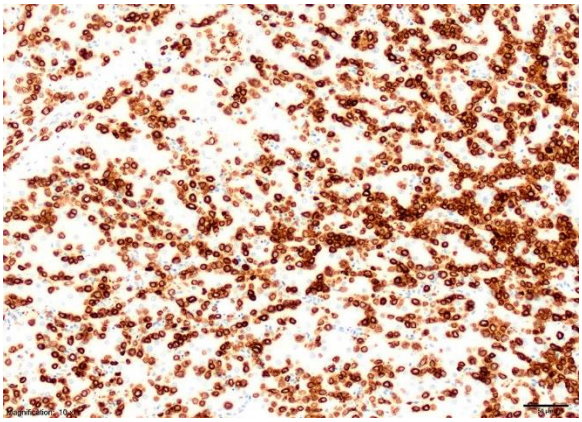
Gross Pathology: The presence of enlarged peripheral nerves and/or visceral lymphomas are commonly seen in Marek's disease, but these lesions are not pathognomonic[9]. Some of the commonly seen lesions include: 1) Enlargement of peripheral nerves (brachial, sciatic and coeliac plexus, abdominal vagus,

and intercostal nerves) with loss of cross-striations and glistening appearance. The affected nerves are edematous and greyish or yellowish in appearance. 2) Development of lymphoid tumors in visceral organs of birds less than 16 weeks of age. 3) Discoloration of the iris and irregularity of the pupil. 4) Lymphomas in the skin around feather follicles.

Microscopic lesions: Affected nerves and visceral tumors contain mixed populations of small to large lymphocytes, lymphoblasts, plasma cells, and macrophages. The peripheral nerves affected in classical and acute forms show three types of lesions: A-type lesions (proliferative type) is characterized by infiltration of proliferating lymphoblasts, small to large lymphocytes, and macrophages; B-type lesion (inflammatory type) is characterized by edema and infiltration of small lymphocytes, plasma cells with proliferation of Schwann cells; C-type lesion (minor infiltrative type) is characterized by mild scattering of small lymphocytes and plasma cells, generally seen in birds with no clinical signs or gross lesions. The proportion of different cell types varies with stage of disease and virulence of the virus with aggressive lymphomas containing of a higher proportion of lymphoblasts.

Immunohistochemistry characteristics: MD tumor cells commonly express MHC-II and T cell markers such as CD4. 5-40% of the tumor cells express MATSA, less than 5% of the cells express IgM. Viral antigens pp38[4] and meq can be detected in tumor cells[5] by immunohistochemistry, fluorescent antibody tests or *in situ* hybridization.[10]

Contributing Institution:
North Carolina State University College of Veterinary Medicine



Liver, peahen. Neoplastic lymphocytes stain strongly positive for CD3. (anti-CD3, 200X) (Photo courtesy of: Kansas State University Veterinary Diagnostic Laboratory/Dept. of Diagnostic Medicine/Pathobiology, http://www.ksvdl.org)

JPC Diagnosis: Liver: Lymphoma, large cell.

JPC Comment: The contributor has done an excellent job in reviewing Marek's disease, one of the most common neoplastic diseases of poultry (and unusual in the fact that it is caused by an alphaherpesvirus (avian alphaherpesvirus-2) rather than a gammaherpesvirus (lymphocryptovirus, rhadinovirus) which is more typical of herpesvirus-driven oncogenesis.)

Joszef Marek (1868-1952) was a noted Hungarian veterinarian, professor of pathology, and ultimately director of the veterinary school in Budapest. In 1907, he first described a peculiar neurological disease which he noticed in his backyard chickens, causing drooping of the wings, and paresis (and ultimately paralysis) of the legs. His multivolume textbook on animal pathology and therapeutics, written at the turn of the century with colleague F. Hutyra, was translated into numerous languages and enjoyed great popularity for decades. He discovered the use of ditrol to control liver flukes, and was awarded the Hungarian Kossuth prize for science in 1949.

Two reports (2016 and 2018) have also described Marek's disease in peafowl – one in the Indian peafowl (*Pavo cristatus*)[2] more commonly known as the "peacock" and one in a green or Javan peafowl (*Pavo muticus)*[8]. Both reports, which describe the pathologic features as well as immunophenotyping of the neoplastic lymphocytes and identification of Marek's disease virus Serotype 1 by PCR, share a number of similarities with this case, including the presence of disease in adult birds.[2,8]

The contributor lists a number of causes of lymphoproliferative disease in poultry, including the common Marek's disease and avian leukosis, the less common avian reticulendotheliosis (a virus that may transform both B and T cells in affected birds), and the relatively unknown retrovirus which causes lymphoproliferative disease in turkeys (with a catchy syndromic name of lymphoproliferative disease in turkeys.)[1,6]

LPDV virus is an exogenous retrovirus which causes lymphoid tumors in some galliforms, especially wild turkeys. The virus was identified first in Europe in the 1970's, and not in North America until 2009.[6] In a study of 800 wild turkeys over a 37 year period,[6] lymphoid neoplasia was seen in approximately 7% of cases, and LPDV virus was identified in over half of those by PCR. In infected birds with neoplastic disease, skin tumors are most common (44%) with liver lesions in 30%. In a few birds in which the lymphocytes were immunophenotyped, the neoplasms appeared to be of T-cell origin, but additional information needs to be performed in this area.[6]

The moderator cautioned on the over interpretation of histologic changes in a single HE slide to differentiated between Marek's disease and lymphoid leukosis. While a positive immunohistochemical test for CD-3 is very helpful in further narrowing the potential diagnosis, definitive diagnosis is still best determined by real-time PCR (performed by the contributor in this case and positive.)

References:

1 Allison AB, Keel MK, Philips JE, Cartoceti AN, Munk BA, Nemeth NM, Welsh TI Thomas JM Crum JM et al. Avian oncogenesis induced by lymphoproliferative dissease virus: a neglected or emerging retrovirual pathogen *Virology* 2014; 450-451:2-12 doi: 10.1016/jvirol 2013.11.037. .

2 Blume GB, Cardoso SP, Oliveira MLB, Maziolli MP, Gomez SYM, Reis Junior JL, Sant Ana FIF, Martins NRS. Visceral Marek's disease in white peafowl (Pavo cristatus). *Arch Bras Med Vet Zootec* 2016:68(6)1602-1608

3 Boodhoo N, Gurung A, Sharif S, Behboudi S: Marek's disease in chickens: a review with focus on immunology. *Veterinary Research* 2016:47(1)**:**119.

4 Gimeno IM, Witter RL, Hunt HD, Reddy SM, Lee LF, Silva RF: The pp38 Gene of Marek's Disease Virus (MDV) Is Necessary for Cytolytic Infection of B Cells and Maintenance of the Transformed State but Not for Cytolytic Infection of the Feather Follicle Epithelium and Horizontal Spread of MDV. *Journal of Virology* 2005:79(7)**:**4545-4549.

5 Nair V: Latency and tumorigenesis in Marek's disease. *Avian Dis* 2013:57(2 Suppl)**:**360-365.

6. Neidringhaus,KD, Nemeth NM, Sellers HS, Brown JD, Fenton HMA. Multicentric round cell neoplasms and their virsal associataions in wild turkeys (Melagris gallopavo) in the southeastern United States. *Vet*

Pathol 2019; doi 10.1177/0300985819864306
7. Pattison PM, Bradbury J, Alexander D. eds., In: *Poultry Diseases*. 6th ed.: Saunders Ltd.; 2008: 258-275.
8. Ranjbar VR, Khordadmehr R. Marke's disease in a peafowl (Pavo muticus); pathological, immunohistochemical and molecular studies. Conference proceedings. *Appr Poultr Dairy Vet Sci:* 2018.
9 Seimon TA, McAloose D, Raphael B, Honkavuori KS, Chang T, Hirschberg D, et al.: A novel herpesvirus in three species of pheasants: Mountain peacock pheasant (Polyplectron inopinatum), Malayan peacock pheasant (Polyplectron malacense), and Congo peafowl (Afropavo congensis). *Veterinary pathology* 2012:49(3)**:**482-491.
10. Swayne DE, ed. Neoplastic Diseases. *Diseases of Poultry*. 13 ed.; 2017.
11. Witter RL: Increased virulence of Marek's disease virus field isolates. *Avian Dis* 1997:41(1)**:**149-163.

CASE II: 2018A (JPC 4134827).

Signalment: 14-day-old, female, SPF chicken (*Gallus gallus*)

History: A 1-day-old chick was inoculated intramuscularly with chicken anemia virus (CAV) that was isolated in Japan in 2017. The bird exhibited petechial hemorrhage of wings on 11 days post inoculation (DPI), depression and dropping wings on 12 DPI, and was found dead on 13 DPI.

Gross Pathology: The bone marrow, spleen and kidney were pale. The thymus was atrophied. The liver was enlarged.

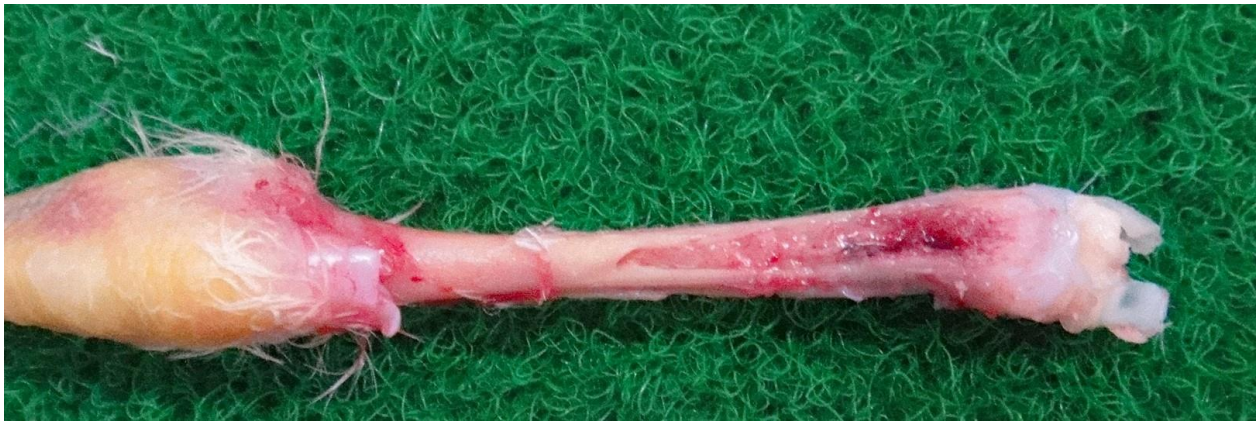
Presentation, chicken. On Day 12 post IM inoculation with chicken anemia agent, the chicken exhibiting petechial hemorrhages and drooping wings. (Photo courtesy of: National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html).

Laboratory results: CAV was reisolated from the liver of the inoculated bird.

Microscopic Description: Bone marrow of tibiotarsus: Hematopoietic cells and erythrocytes were significantly decreased and were replaced by loose connective tissues. Low number of the large atypical cells were scattered in the extravascular spaces. Some of the atypical cells contained one to a few small eosinophilic nuclear inclusion bodies.

Contributor's Morphologic Diagnosis:
Bone marrow: Hypoplasia, severe, with occasional large atypical cells with small eosinophilic intranuclear inclusion bodies.

Contributor's Comment: The chicken anemia virus (CAV) was first detected in Japan in 1979.[18] CAV currently belongs to the *Gyrovirus* genus, Anelloviridae family.[9] CAV is a non-enveloped, icosahedral virus measuring about 19 nm in diameters, with a circular, single-stranded DNA genome.[4,13,18]



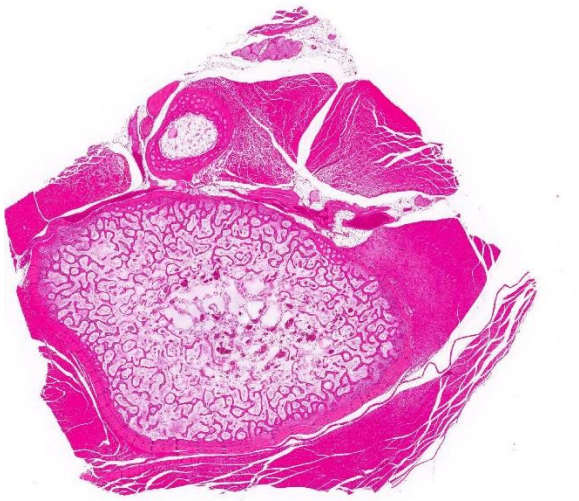
Femur, chicken. The bone marrow is extremely pale. (Photo courtesy of: National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html).

Chickens are considered only natural hosts of CAV. CAV is ubiquitous in flocks around the world, and most flocks carry antibodies regardless of vaccination.[10-12,20]

CAV is mainly transmitted vertically through eggs and causes anemia, anorexia and lethargy to chicks.[19,21] Hematocrit values decline less than 10% of normal in severe cases.[3,16] Infection with CAV alone in chickens of 2-week-old or older is subclinical.[16] If clinical signs develops in these chicks, immune depression is suspected.[6]

CAV targets hemocytoblasts in the bone marrow and T lymphoblasts in the thymus cortex, leading to hypoplasia of the bone marrow and atrophy of the thymus lobules.[1,2] Damage to the bone marrow and the lymphatic tissues causes anemia, circulatory failure and low platelets, and which develop the pale colored viscera and hemorrhagic lesions. The liver swelling and atrophy of the bursa of Fabricius can also be seen.[17,18]

Histologic findings include depletion of hematopoietic tissues in the bone marrow and depletion of lymphocytes in the lymphoid tissues.[17] Eosinophilic intranuclear inclusion bodies can be observed in the bone marrow and the thymus of the infected birds.[5] The inclusion bodies are occasionally detected in the spleen, proventriculus, lung, kidney, bursa of Fabricius and skin.[15] CAV infection needs to be differentiated from infectious bursal disease (IBD). Infection of highly pathogenic IBD virus make lesions similar to CAV infection in lymphoid tissues, bone marrow and skeletal muscle.[7,8,14] However, in IBD infection, inclusion bodies are not observed.



Tibia and fibula, chicken. The bone marrow is diffusely and severely hypoplastic. (HE 9X)

Contributing Institution:
National Institute of Animal Health,
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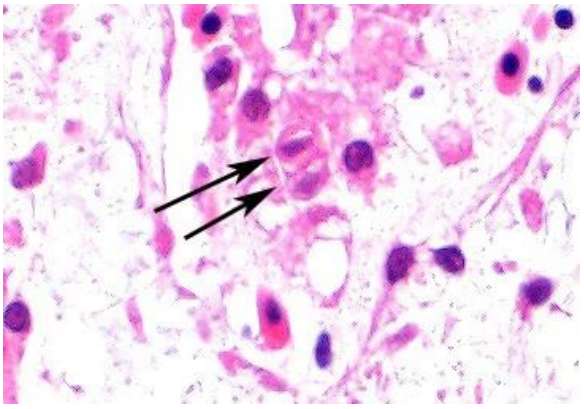
(WSC ID95)
http://www.naro.affrc.go.jp/english/niah/index.html

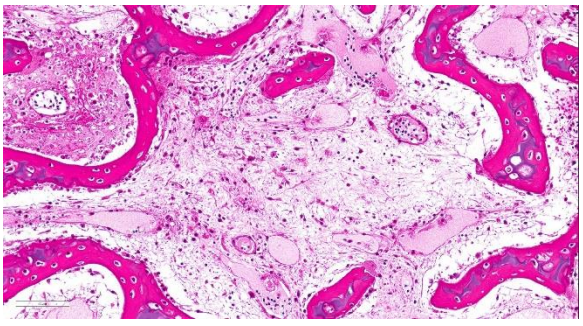
JPC Diagnosis: 1. Tibial bone marrow: Necrosis and atrophy, diffuse, severe, with edema and rare hemocytoblastic and osteoclastic intranuclear inclusions.



Tibial bone marrow, chicken. Few remaining hematoblasts contain large rhomboid intranuclear inclusions (arrows).

JPC Comment: Chicken anemia agent is a major cause of immunosuppression in young chickens on a global basis, and is often associated with other diseases, such as necrotic dermatitis, hemorrhagic syndrome, aplastic anemia syndrome, and blue wing disease.

This slide is an excellent representation of the changes noted in experimental infections with this agent. Following injection with a virulent strain of CAV, bone marrow cellularity begins to decrease approximately between 4-8dpi.[5,15] Large hematopoietic cells, larger than normal pre-erythroblasts are present within the intra- and extravascular spaces. These cells frequently contain intranuclear inclusions, as do degenerating hematopoietic cells. From days 12-20, (the time period from which this particular slide was collected), there is severe depletion of both erythropoietic and granulocytic cells. Inclusions may be more common seen in osteoclasts during this period, as few hemocytic precursors remain.[5] Similar changes proceed apace along this timeline within the thymus, bursa, and splenic white pulp as well. Intranuclear inclusions may be seen within lymphocytes in all of these tissues.[15]



Tibial bone marrow, chicken. Higher magnification of the tibial bone marrow demonstrating the absolute lack of progenitor cells and equally few erythrocytes within blood vessels. (HE 350X).

In surviving birds, the bone marrow and lymphoid begin to repopulate by day 16dpi and day 24 dpi, with tissue recovery by day 32dpi.[15] In one study, 35 of 50 (70%) of inoculated birds succumbed to the disease, mostly between days 14 and 18.[17]

The importance of infection with chick anemia agent is far more than early and permanent immunosuppression in chicks infected within the first few days of life. Earlier in the day, the moderator had presented a lecture on immunosuppressive disease suggesting that a second wave of immunosuppression may be seen in animals infected with chick anemia agents at approximately 18 days of age due to profound thymic atrophy. If injected later, it may also potentiate the effects of other

immunosuppressive agent, such as IBD, resulting in significant immunosuppression and related diseases (reovirus, respiratory disease, clostridiosis, E. coli, coccidosis) in affected birds from 18-35 days.

The assembled participants discussed the difficulty of discerning the intranuclear inclusions in this disease. There was general agreement that they were in hemocytoblasts and osteoclasts, but agreement on what were inclusions and what were degenerating nuclei was more difficult to come by.

References:

1. Adair BM, McNeilly F, McConnell CD, McNulty MS. Characterization of surface markers present on cells infected by chicken anemia virus in experimentally infected chickens. *Avian Dis.* 1993; **37**:943-950.

2. Adair BM. Immunopathogenesis of chicken anemia virus infection. *Dev Comp Immunol.* 2000; **24**:247-255.

3. Campbell TW, Ellis C. Appendices. In: *Avian & exotic animal hematology & cytology.* 3rd ed. Oxford, UK: Blackwell Publishing; 2007:246.

4. Goryo M, Suwa T, Matsumoto S, Umemura T, Itakura C. Serial propagation and purification of chicken anaemia agent in MDCC-MSBI cell line. Avian Pathol. 1987; **16**:149-163.

5. Goryo M, Suwa T, Umemura T, Itakura C, Yamashiro S. Histopathology of chicks inoculated with chicken anaemia agent (MSB1-TK5803 strain). *Avian Pathol.* 1989; **18**:73-89.

6. Imai K, Mase M, Tsukamoto K, Hihara H, Yuasa N. Persistent infection with chicken anaemia virus and some effects of highly virulent infectious bursal disease virus infection on its persistency. *Res Vet Sci.* 1999; **67**:233-238.

7. Inoue M, Fukuda M, Miyano K. Thymic lesions in chicken infected with infectious bursal disease virus. *Avian Dis.* 1994; **38**:839-846.

8. Inoue M, Fujita A, Maeda K. Lysis of myelocytes in chickens infected with infectious bursal disease virus. *Vet Pathol.* 1999; **36**:146-151.

9. International committee on taxonomy of viruses https://talk.ictvonline.org/ictv-reports/ictv_9th_report/ssdna-viruses-2011/w/ssdna_viruses/139/anelloviridae

10. McNulty MS, Connor TJ, McNeilly F, Kirkpatrick KS, McFerran JB. A serological survey of domestic poultry in the United Kingdom for antibody to chicken anaemia agent. *Avian Pathol.* 1988; **17**:315-324.

11. McNulty MS, Connor TJ, McNeilly F. A survey of specific pathogen-free chicken flocks for antibodies to chicken anaemia agent, avian nephritis virus and group a rotavirus. *Avian Pathol.* 1989; **18**:215-220.

12. McNulty MS, Connor TJ, McNeilly F, Spackman D. Chicken anemia agent in the United States: isolation of the virus and detection of antibody in broiler breeder flocks. *Avian Dis.* 1989; **33**:691-694.

13. McNulty MS, Curran WL, Todd D, Mackie DP. Chicken anemia agent: an electron microscopic study. Avian Dis. 1990; **34**:736-743.

14. Nunoya T, Otaki Y, Tajima M, Hiraga M, Saito T. Occurrence of acute infectious bursal disease with high mortality in Japan and pathogenicity of field isolates in specific-pathogen-free chickens. *Avian Dis.* 1992; **36**:597-609.

15. Smyth JA, Moffett DA, McNulty MS, Todd D, Mackie DP. A sequential histopathologic and immune-cytochemical study of chicken anemia virus infection at one day of age. *Avian Dis.* 1993; **37**:324-338.

16. Takagi S, Goryo M, Okada K. Pathogenicity for chicks of six isolation of chicken anemia virus (CAV) isolated in the past two years ('93 '94). *J Jpn Soc Poulf Dis.* 1996; **32**:90-97.

17. Taniguchi T, Yuasa N, Maeda M, Horiuchi T. Hematopathological changes in dead and moribund chicks induced by chicken anemia agent. *Natl Inst Anim Health Q (Tokyo).* 1982; **22**:61-69.

18. Yuasa N, Taniguchi T, Yoshida I. Isolation and some characteristics of an agent inducing anemia in chicks. *Avian Dis.* 1979; **23**: 366-385.

19. Yuasa N, Yoshida I. Experimental egg transmission of chicken anemia agent. *Natl Inst Anim Health Q (Tokyo).* 1983; **23**:99-100.

20. Yuasa N, Imai K, Tezuka H. Survey of antibody against chicken anaemia agent (CAA) by an indirect immunofluorescent antibody technique in breeder flocks in Japan. *Avian Pathol.* 1985; **14**:521-530.

21. Yuasa N, Imai K. Pathogenicity and antigenicity of eleven isolates of chicken anaemia agent (CAA). *Avian Pathol.* 1986; **15**:639-645.

CASE III: 2018A (JPC 4135750).

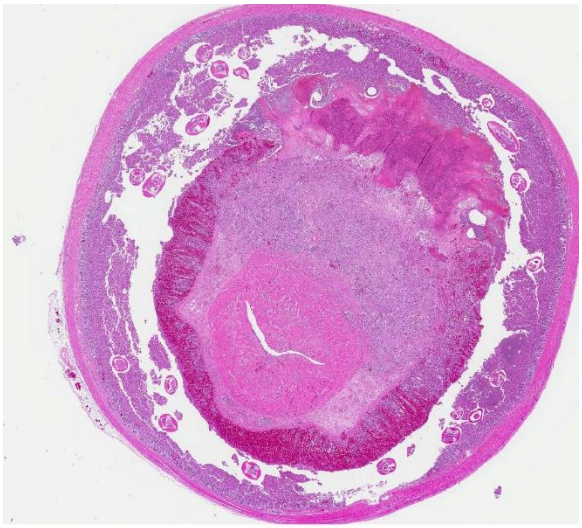
Signalment: 9-month-old, Ancona hen, *Gallus gallus domesticus*

History: One hen in a flock of 12 was euthanized following a 7-days history of ataxia progressing to unilateral paresis.

Gross Pathology: The bone marrow, spleen and kidney were pale. The thymus was atrophied. The liver was enlarged.

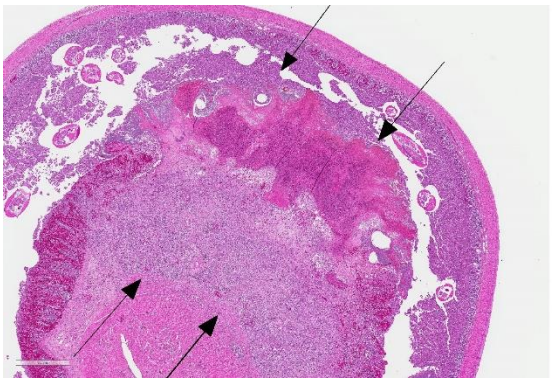
Laboratory results: None performed.

Microscopic Description: At the level of the grossly identified cecal intussusception, the mucosa and submucosa of the intussusceptum are segmentally disrupted by lytic necrosis and granulomatous inflammation. In the areas of necrosis, the mucosa is replaced by a large aggregate of cellular and nuclear debris, fibrin, myriad mixed bacteria, hemorrhage



Cecum, chicken. A segment of cecum has telescoped into another, resulting in severe congestion and mucosal hemorrhage within the intussuscipiens. Within the lumen of the intussusceptum, there are numerous cross sections of adult ascarids. (HE, 12X)

and several weakly eosinophilic protozoal trophozoites (histomonads). Trophozoites are round, 7-17 µm diameter, with a single, central, 3 µm diameter nucleus. The underlying lamina propria and submucosa are expanded by abundant macrophages, multinucleated giant cells (foreign body and Langhans-type), lymphocytes, plasma cells, few heterophils, plump fibroblasts (fibrosis), tortuous capillaries (neovascularization), and extracellular and intrahistiocytic trophozoites. The remaining mucosa of the intussusceptum is diffusely expanded by hemorrhage and superficial enterocytes are sloughed. The cecal lumen contains numerous cross and tangential sections of adult, female nematodes and few nematode eggs. The adult nematodes have a thin smooth cuticle, lateral alae, lateral cords, coelomyarian musculature, and a psuedocoelom containing a digestive tract lined by columnar cells with a brush border, a simple esophagus, an ovary, a muscular vagina and a uterus containing ova in various stages of development and few sperm. Intraluminal nematode eggs are oval and ~40 x 60 µm, with a thick smooth shell surrounding a uninucleate zygote. The mucosa of the examined sections of the intussuscipiens is multifocally attenuated, disrupted by autolysis, and the lamina propria is infiltrated by moderate to abundant lymphocytes and plasma cells.

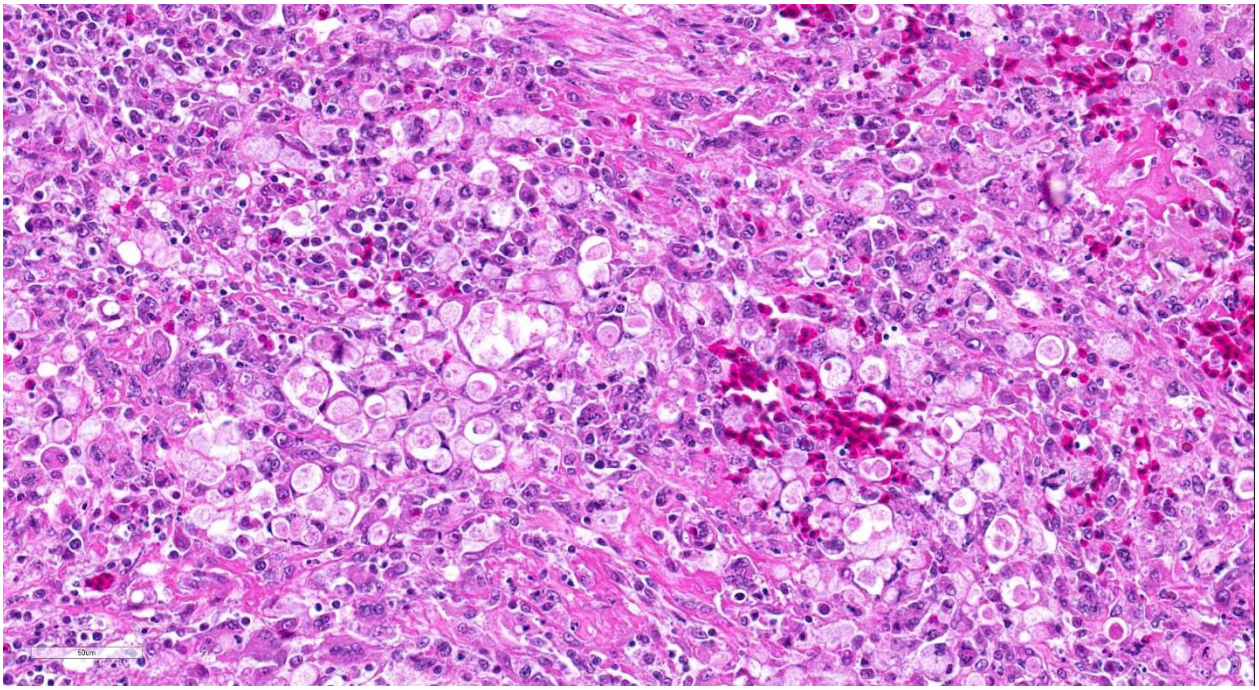


Cecum, chicken. Segmentally, within the intussuscipiens, there is an extensive area of lytic necrosis within the mucosa which expands the submucosa (arrows). (HE, 23X)

Other findings: Lymphocytic meningitis and peripheral neuritis, consistent with Marek's disease.

Contributor's Morphologic Diagnosis:
Cecal intussusception and typhlitis, granulomatous and necrotizing, segmental, subacute to chronic, severe, with adult nematodes (consistent with *Heterakis gallinarum*) and protozoal trophozoites (consistent with *Histomonas meleagridis*)

Contributor's Comment: Blackhead disease (histomoniasis) is caused by the protozoan flagellate *Histomonas meleagridis*. Classically this parasite is transmitted when susceptible species (i.e. gallinaceous birds, ducks, geese, game birds, and zoo birds) ingest *H. meleagridis*-infected ova or adults of the intermediate host, *Heterakis gallinarum* (cecal worm of poultry). In addition to this mode of transmission, turkeys can acquire infection through cloacal contact with contaminated feces and retrograde transport of histomonads to the ceca; a process known as cloacal drinking.[2] Once in the ceca, *H. meleagridis* can invade the cecal mucosa and cause local inflammation and necrosis, as seen in this case. However, lesions only develop if the ceca are co-colonized by certain types of bacteria (e.g. *E. coli*, *Clostridium perfringens*, *Bacillus subtilis*, *Salmonella* sp.). The importance of bacteria in the pathogenesis of histomoniasis has been demonstrated in studies showing that *H. meleagridis* is avirulent in germ-free ceca[6] and gnotobiotic turkeys.[1] In some birds, histomonads subsequently invade the portal circulation and spreads to the liver to cause

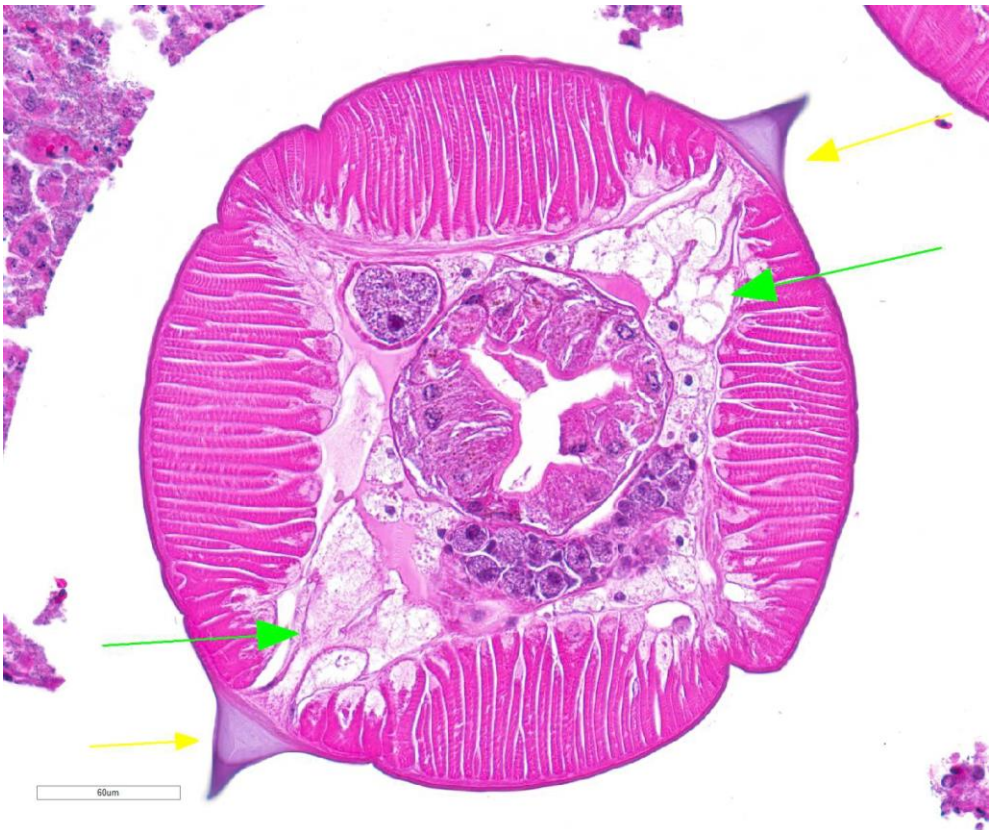


Cecum, chicken. Within the submucosa numerous histomonads are present within macrophages and occasionally free within the inflammatory infiltrate. (HE, 400X)

necrotizing and fatal hepatitis. This was not observed in the present case and the clinical signs that led to euthanasia were attributed to concurrent Marek's disease.

Turkeys are highly susceptible to developing systemic histomoniasis, with mortality rates reaching 100% in some flocks.[8] Conversely, in chickens, *H. meleagridis* causes relatively low mortality (10-20%) and high to low morbidity characterized by decreased egg production, and decreased weight gain.[1,8] This difference in susceptibility is not fully understood, but current data indicate that innate and adaptive immune responses play an important role.[9] In chickens, histomonads elicit an innate immune response (i.e. increased expression of IL-1β, CXCLi2, and IL-6) in the cecal tonsils within the first 24 hours of infection.[10] Turkeys, however, do not upregulate these pro-inflammatory cytokines until the organism is detectable in the liver.[10] In addition, one study of the adaptive immune response demonstrated that chickens have a higher percentage of IFN-ɣ mRNA expressing cells in the cecum prior to infection, suggesting these cells may play a protective role in histomoniasis.[7] In contrast, the number of IFN-ɣ positive cells in the ceca of unvaccinated turkeys initially decrease following infection and then increase coincident with cecal inflammation and necrosis. It is thought that the intensity of these delayed immune responses is an important contributor to the severity of disease in turkeys.[7,10]

Management of histomoniasis in commercial and backyard flocks has become more difficult in recent years because the last drug approved for the prevention of blackhead disease was disallowed in the U.S. in 2016.[1] Several alternative therapies are currently being investigated, but none have been proven to be sufficiently efficacious for broad application and a protective vaccine is not available.[1] Therefore, traditional environmental management practices (e.g.



Cecum, chicken. Cross-sections of adult nematodes are present within the luminal debris. This adult female has a thin cuticle with lateral alae (yellow arrows), prominent lateral chords (green arrows), tall coelomyarian/polymyarian musculature, an intestine with tall columnar epithelium and a brush border, and numerous cross sections of the uterus with developing eggs. (HE, 253X)

biosecurity, management of soil and litter, avoid comingling highly susceptible species with chickens, etc.) are of increasing importance in the prevention of this disease.

Contributing Institution:
Washington Animal Disease Diagnostic Laboratory (WADDL); Department of Veterinary Microbiology and Pathology, Washington State University.
https://waddl.vetmed.wsu.edu/
https://vmp.vetmed.wsu.edu/

JPC Diagnosis: 1. Cecum: Intussuception.
2. Cecum: Typhlitis, necrohemorrhagic and granulomatous, transmural, multifocal to coalescing, marked with numerous amebic trophozoites.
3. Cecum, lumen: Adult ascarids, multiple.

JPC Comment: With the recent removal of nitarsone, the last remaining feed additive targeting flagellates, the economic importance of *Histomonas meleagridis* is greater than ever before. A recent retrospective of the disease in commercial turkeys was published in 2018[4], in concert with similar retrospective studies in France and Germany. In the California study, most cases occurred in warmer months from April to October, likely due to the longer survival of trophozoites outside the host during these months. Affected birds ranged from 2 weeks to 15 months; and the disease is less frequent, but no less severe in older birds. In most autopsied birds, histomoniasis was considered the primary cause of death. Histomonads were observed outside of the cecum and liver in 12/66 cases; other affected organs included spleen kidney, bursa, proventriculus, pancreas, lung, and crop. In five out of the 66 cases, the infection spread to all houses in the facility but cecal worms were only seen in 2 out of 66 cases, suggesting other forms of spread and the possibility of more resistant forms.[4]
H. meleagridis has recently been characterized in peafowl.[2] They may be

experimentally infected with *H. meleagridis*, but natural infections are rare. In a recent retrospective of infected peafowl, characteristic gross and histologic lesions associated with *H. meleagridis* (necrotizing typhlitis and hepatitis) were noted in each case, however, no birds had concurrent *H. gallinarum* infection. One bird had a concurrent infection with *Tetratrichomonas gallinarum*; the significance of this infection in facilitating the histomonad infection (seen with a number of other bacteria as mentioned by the contributor) is yet unclear.[2]

Interestingly, in a retrospective of common mortality in commercial egg-laying chickens, intussusception (classified along with volvulus under "twisted intestine") ranked seventh in the top fifteen causes of normal mortality at 3.5% of 3337 necropsies. Intussusception ffis reported to occur frequently secondarily to coccidiosis, necrotic enteritis, or intestinal parasitism, although in that particular study, intestinal parasites were not found. Affected birds demonstrate emaciation and atrophy of the reproductive tract, suggesting that the lesions are generally chronic in nature.[3] The moderator discussed the possibility of a number of cases of intussusception seen in non-parasitized birds perhaps being the result of skipping a feeding day in pullets, similar to the so-called "re-feeding syndrome" seen in humans.

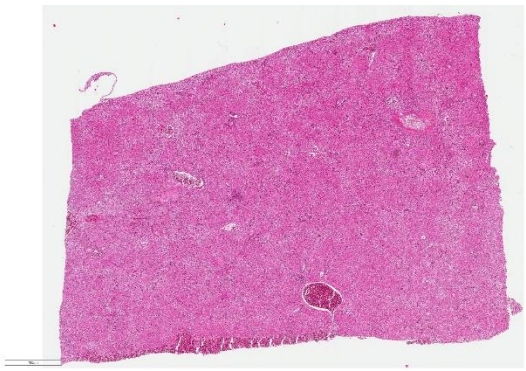
References:

1. Clark S, Kimminau E. Critical Review: Future Control of Blackhead Disease (Histomoniasis) in Poultry. *Avian Dis*. 2017;61**:** 281-288.
2. Clarke LL, Beckstead BM, Hayes JR, Rissi D. Patholoic and molicular charcterization of histomoniasis in peafowl (Pavo cristatus). *J Vet Diagn Investig* 2017; 29(2):237-241.
3. Fulton RM. Causes of normal mortality in commercial egg-laying chickens. *Avian Dis* 2017; 61(3): 289-295.
4. Hauck R, Stoute S, Chin RP, Senties-Cue CG, Shivaprasad HL. Retrospective sutdy of histomoniasis (blackhead) in California turkey flocks 2000-2014.
5. Hu J, Fuller L, McDougald LR. Infection of turkeys with Histomonas meleagridis by the cloacal drop method. *Avian Dis*. 2004;48**:** 746-750.
6. Kemp RL. The failure of Histomonas meleagridis to establish in germ-free ceca in normal poults. *Avian Dis*. 1974;18**:** 452-455.
7. Kidane FA, Mitra T, Wernsdorf P, Hess M, Liebhart D. Allocation of Interferon Gamma mRNA Positive Cells in Caecum Hallmarks a Protective Trait Against Histomonosis. *Front Immunol*. 2018;9**:** 1164.
8. McDougald LR. Blackhead disease (histomoniasis) in poultry: a critical review. *Avian Dis*. 2005;49**:** 462-476.
9. Mitra T, Kidane FA, Hess M, Liebhart D. Unravelling the Immunity of Poultry Against the Extracellular Protozoan Parasite Histomonas meleagridis Is a Cornerstone for Vaccine Development: A Review. *Front Immunol*. 2018;9**:** 2518.
10. Powell FL, Rothwell L, Clarkson MJ, Kaiser P. The turkey, compared to the chicken, fails to mount an effective early immune response to Histomonas meleagridis in the gut. *Parasite Immunol*. 2009;31**:** 312-327.

CASE IV: S699/14 (JPC 4085966).

Signalment: Hessian crop pigeon, juvenile (< 1 year), weight: 440 g, female

History: The examined animal came from a private breeding livestock. Several pigeons



Liver, pigeon: A section of liver is submitted for examination. At low magnification, a retiform pattern of hepatic glycogenosis is evident upon close inspection as are scattered foci of hemorrhage. (HE, 30X)

died without any clinical symptoms. Treatment was not applied.

Gross Pathology: Both liver and kidneys showed multifocal beige to dark-brown colored foci, in diameter 0.2-0.3 mm, extending to subjacent parenchyma. In the lungs multifocal moderate acute hemorrhages were diagnosed. The pigeon was in good body condition.

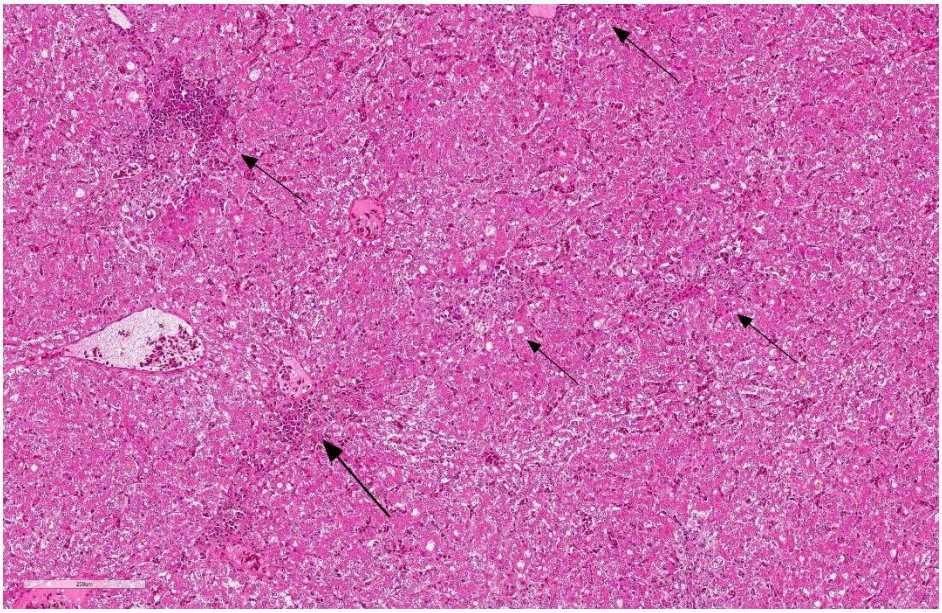
Laboratory results: Molecular biological examination:
PCR for Pigeon Herpesvirus (PiHV): positive
PCR for Pigeon Circovirus (PiCV): positive

Parasitological examination:
Detection of a small number of coccidial oocysts

Microscopic Description: Liver: There are multifocal areas of acute necrosis, in some areas associated to the portal fields. These areas are demarcated by a moderate infiltration with predominantly histio-cytes/macrophages, some lymphocytes and a few neutrophils and plasma cells and a very mild beginning fibrosis. In numerous intralesional hepatocytes large (5-10 µm) amphophilic to basophilic intranuclear inclusion bodies type Cowdry A can be detected. Partly these large inclusions possibly seem to be located in the cytoplasm of the hepatocytes as well. Furthermore numerous hepatocytes show intranuclear eosinophilic inclusion bodies (ca. 3 µm) type Cowdry B. There is a diffuse moderate irregularity and dissociation of the cords of hepatocytes with a diffuse moderate anisocytosis and anisokaryosis of the hepatocytes. Multifocal a mid-zonal to peripheral lobular localized degeneration of hepatocytes is detected. In the periphery of areas of necrosis a few round and lightly basophilic structures (councilman-bodies) can be noticed.
With reference to the portal fields a mild to moderate hyperplasia of bile ducts and a very mild interstitial fibrosis with fibroplasia is obvious. Additionally, in the cytoplasm of several hepatocytes and Kupffer cells a small amount of light brown to green and of golden-yellow to light green fine and coarse granules is detected, most likely consistent with a mild hemosiderosis and a mild storage of bile pigment. Diffuse moderate hyperemia and multifocal mild acute hemorrhages are obvious.

Contributor Morphologic Diagnosis:
Liver: hepatitis, necrotizing and histiocytic/granulomatous, multifocal, polyphasic, moderate with numerous intranuclear inclusion bodies.

Contributor's Comment: Circoviruses are very small (15-20 nm in diameter) non-enveloped icosahedral viruses with circular single-stranded DNA. Because of their small size circoviruses are dependent on cellular enzymes of the host for their replication. Therefore, tissues with rapid cell proliferation, such as lymphoid tissue, are affected by circovirus.[2,7] Beside the pigeon circovirus (PiCV) three other circoviruses are known to be infectious pathogens of



Liver, pigeon: Randomly scattered throughout the section are numerous foci of necrosis in which hepatocytes are individualized, shrunken, and hypereosinophilic. Nuclei of hepatocytes at the periphery often contain a single intranuclear viral inclusions surrounded by a clear halo. (HE, 146X)

spontaneous diseases. Chicken anemia virus (CAV) in fowl, which has been reclassified in the genus Gyrovirus, psittacine beak and feather disease (PBFD) in psittacine birds and porcine circovirus (PCV) in pigs.[4]

The PiCV was first identified in the USA but since it has been reported in many European countries, in North America, Australia and South Africa, probably a worldwide distribution is assumed. The practice of the pigeon sports probably supported the worldwide spread of the virus.[2]

Typically PiCV infects, as in the present case, young pigeons under one year of age. The way of transmission is not fully understood yet, but most infections seem to occur an oral way. Additionally, an egg-transmitted infection is also to be taken in consideration.[5]

Clinical signs can be lethargy, growth retardation, poor race performance and various symptoms caused by secondary bacterial or parasitic infections induced by immunosuppression. In contrast to PBFD a dystrophy and loss of feathery or beak deformations can only be seen rarely in PiCV infections. PiCV is normally associated with high morbidity and varying mortality, depending on secondary infections. In necropsy gross findings are usually very rare, atrophy of the bursa of Fabricius can occur. Other lesions often result from the secondary infections. A common histopathological finding is necrotizing bursitis and large (up to 15µm) basophilic intracytoplasmic inclusion bodies in the cells of the lymphoid follicles. Rarely these inclusion bodies are seen in other organs, such as spleen, thymus and GALT/BALT.[1,2,4,5] Definitive diagnosis can be made by PCR or in situ hybridisation. The Pi(CV) is found in the bursa of Fabricius, spleen, thymus, kidney, respiratory system and liver. As PiCV can be detected in clinically and histopathologically normal pigeons, many PiCV infections seem to be subclinical.[5]

The young pigeon disease syndrome (YPDS, swollen gut syndrome) is a multifactorial disease in young pigeons, aged from 4 to 12 weeks. Its etiological agent(s) is (are) still unknown. The PiCV plays an important role in YPDS by inducing immunosuppression. Other possibly involved infectious agents are pigeon herpesvirus, pigeon adenovirus, avian polyomavirus and bacterial pathogens such as *Spironucleus columbae* and *Escherichia coli*.[5]

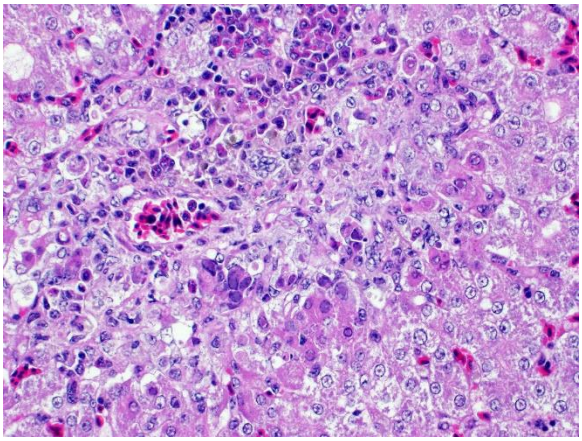
Herpesviruses are large (180-250 µm in diameter) enveloped viruses with double-stranded DNA and an icosahedral capsid. Their replication occurs within the nucleus of the host cell.

Pigeon herpesvirus-1 (PiHV-1) has a worldwide distribution. Pigeons are the natural hosts of PiHV-1, in which it remains latent. Adult pigeons are asymptomatic carriers. Squabs are infected very early in life by latently infected pigeons feeding the squabs with cropmilk. The squabs are protected if they received maternal antibodies conferred with egg yolk. These pigeons also become asymptomatic carriers. In case the egg yolk does not contain any maternal antibodies, the emerging squabs are fully susceptible for PiHV-1 infection and develop clinical symptoms.

Adult pigeons (latent carriers) often appear completely healthy, although PiHV-1 can be isolated from the pharynx of these birds. Susceptible young birds develop clinical symptoms. In necropsy retarded development and pharyngitis, partly associated with small foci of necrosis and small ulcers are observed. In generalized infections the histopathological examination reveals necrosis in the liver and the spleen. Intranuclear inclusion bodies in hepatocytes are common findings.

Infection with *Trichomonas gallinae*, *Chlamydia* spp. or avian paramyxovirus should be considered as differential diagnoses.

Serological examinations (in positive cases) can only provide information about the infection state of the animal. Diagnosis of PiHV-1 infection should be confirmed by virus isolation.[3,9]



Liver, pigeon: Areas of necrosis are infiltrated by moderate numbers of heterophils (top) and macrophages. Nuclei of hepatocytes at the periphery often contain intranuclear viral inclusions. (HE, 400X)

Contributing Institution:
Institut für Veterinär-Pathologie, Veterinärmedizinische Fakultät Leipzig, Universität Leipzig, Germany (http://www.vetmed.uni-leipzig.de/ik/wpathologie)

JPC Diagnosis Liver: Hepatitis, necrotizing, random, multifocal, subacute, with small eosinophilic and large basophilic intranuclear viral inclusions.

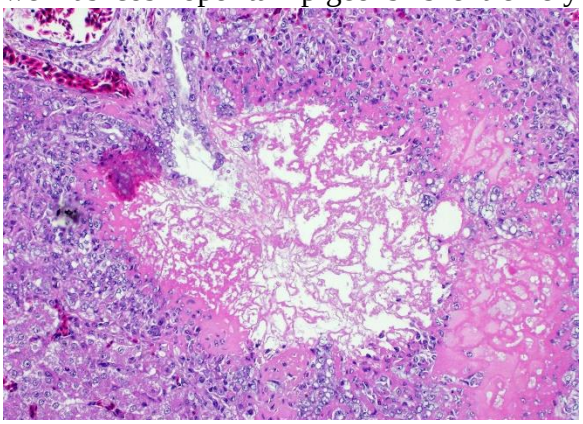
JPC Comment: The contributor does an excellent job at describing two common viral infections in pigeons, only one of which can be diagnosed by examining this particular slide.

Like most alphaherpesviruses, the course of herpesviral disease is most often subclinical in the natural host, but may cause significant damage is other species. In the pigeon, most infections are latent, but may recrudesce in times of stress or concurrent disease. In Belgium, 50% of pigeons have antibodies to the virus; when affected with other respiratory pathogens, PHV-1 can be isolated in the pharynx of up to 80% of birds.[4] In adult birds, clinical disease is limited to the respiratory tract with necrotizing lesions in

the mouth, pharynx and larynx; superficial bacterial lesion results in more severe lesions.[9] Necrotizing hepatitis is most often seen in young birds which did not receive maternal immunity through the egg yolk, making this an intriguing potential cause of "young pigeon disease syndrome".

The disease in pigeons, is eclipsed by the disease in owls and falcons who have preyed upon infected pigeons. PCR studies have demonstrated that the herpesvirus that causes "hepatosplenitis infectiosa strigum" or "hepatosplenitis" in raptors is identical to columbid-herpesvirus 1, once again illustrating the severe disease which often results when alphaherpesviruses jump into aberrant hosts. For more information on the disease in raptors, see the following WSC cases – WSC 2017-2018 Conf 15 Case 1, WSC 2014-2015 Conferenc 13, case 4, WSC 2010-2011, Conference 8, Case 1.

Pigeon circovirus (PiCV) was first diagnosed in Canada in 1986, and shortly after, was diagnosed in many countries around the world. In recent studies, infection rates in flocks range averaged around between 75 and 80% in China and Europe, with similar rates in healthy and unhealthy birds. [6] The potential transmission of this disease in racing birds as well as cosmopolitan pigeons is extremely high. While mortality in affected flocks in two-month- to 1-year-old pigeons may be up to 100%[9], the potential of circoviruses to cause lymphoid necrosis and immunosuppression in affected birds may be even more profound.[9]



Liver, pigeon. Areas of necrosis extending into portal areas breach the biliary epithelium, resulting in release of bile into the surrounding parenchyma and liquefactive necrosis. (HE, 400X)

"Young pigeon disease syndrome", (YPDS) as mentioned by the contributor, is a syndrome of disease which appears closely interrelated with the immunosuppressive effects of pigeon circovirus, much like the many syndromes associated with porcine circovirus-2. It was first described in the 1980s, and affects birds from 4-12 weeks of age, resulting in anorexia, ruffled feathers, regurgitation and diarrhea, excessive crop liquid, and polyuria. In affected flocks, morbidity approaches 20% and mortality 50%. As the contributor has stated, a variety of infectious agents, in addition to PiCV have been incriminated, to include pigeon adenoviruses and herpesviruses. A recent study of birds with YPDS in Poland demonstrated a 44.5-100% infection rate with PiCV, and the second most common viruses in affected birds was pigeon herpesvirus-1, at approximately 30%.[7] Pigeon adenovirus was not identified in any flocks in this study.

The characteristic botryoid basophilic intracytoplasmic inclusions of pigeon circovirus are largely restricted to lymphoid tissues, and would not likely be seen within hepatocytes in this case, although there was great variation in size and shape of the inclusions in this case. While the age of this bird may or may not be consistent with the syndrome of YPDS, and the evidence is circumstantial at best, it is interesting to ponder the effects of PiCV in generating clinical systemic herpesviral disease in a mature bird.

References:

1. Abadie J, Nguyen F, Groizeleau C, Amenna N, Fernandez B, Guereaud C, Guigand L, Robart P, Lefebvre B, Wyers M: Pigeon circovirus infection: Pathological observations and suggested pathogenesis. Avian Pathology. 2001; 30(2): 149-158

2. Duchatel JP, Szeleszcuk P: Young pigeon disease. Medycyna Wet. 2011; 67(5): 291-294

3. Kaleta EF Herpesvirus der Tauben. In: Kaleta EF and Krautwald-Junghanns M-E ed. Kompendium der Ziervogelkrankheiten. Hannover: Schlütersche GmbH & Co. KG Verlag und Druckerei; 1999; 292-294

4. Marlier D, Vindevogel H: Viral infections in pigeons. The veterinary journal. 2006; 172: 40-51

5. Raue R, Schmidt V, Freick m, Reinhardt B, Johne R, Kamphausen L, Kaleta EF, Müller H, Krautwald-Junghanns M-E: A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. Avian Pathology. 2005; 34(5): 418-425

6. Stenzel TA, Pestka D, Tyklowski B, Smialek M, Knocicki A. Epidemiological investigation of selected pigeon viral infections in Poland. *Vet Rec*

7. Todd T: Circoviruses: immunosuppressive threats to avian species. Avian Pathology. 2000; 29: 373-394

8. Todd T: Avian circovirus diseases: lessons for the study of PMWS. Veterinary Microbiology. 2004; 98: 169-174

9. Vindevogel H, Duchatel JP. Pigeon Herpesvirus Infection. In: Calnek BW, Barnes HJ, Beard CW, Reid WM, Yoder Jr. HW ed. Diseases of Poultry. Ninth Edition. Ames: Wolfe Publishing Ltd; 1991; 665-667