



WEDNESDAY SLIDE CONFERENCE 2026-2027

Conference #8

14 October 2026

CASE I:

Signalment:

6-month-old, intact male, Maine Coon, *Felis catus*, feline

History:

This cat had an acute onset of lethargy and anorexia. The cat was presented to a small animal emergency service for neurologic signs consistent with obtundation. On presentation, the eyes were bilaterally sunken and third eyelids were protruding. Further examination revealed slightly increased bronchovesicular

sounds, elevated body temperature, and ambulatory ataxia. During hospitalization, the cat experienced acute respiratory arrest followed by cardiac arrest.

Gross Pathology:

At necropsy, the lungs were soft, diffusely mottled red to dark red, and oozed dark red, opaque fluid on cut section. The ileocecal lymph node was moderately enlarged and measured approximately 2.5 cm x 1.5 cm x 1.5 cm. There were no other significant gross lesions observed.

Laboratory Results:

Michigan State University Veterinary Diagnostic Laboratory

RT-PCR

Avian Influenza virus matrix PCR

Nasal swab - Non-negative (AIV 31.91 CT)

Avian Influenza Virus H5 PCR

Nasal swab - Negative (AIV 32.39 CT)

Avian influenza Virus H5 2.3.4.4 PCR

Nasal swab - Non-negative (AIV 33.02 CT)

Immunohistochemistry

Cerebrum - AIV Antigen Detected

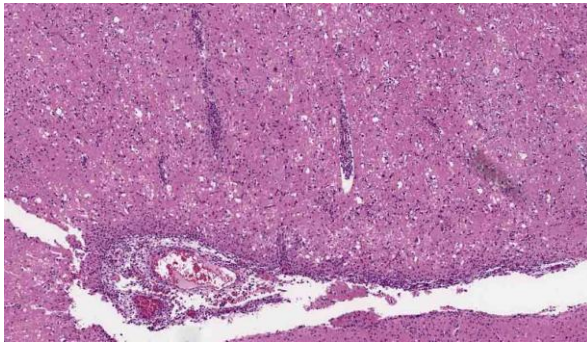
Lung - AIV Antigen Not Detected

Michigan Department of Health and Human Services

Indirect fluorescent antibody test, brain: Rabies negative



1-1. Cerebrum, cat: Two sections of the cerebrum are submitted for examination; one section (top) is from the telencephalon. In the second section (bottom), corpus is from the diencephalon and contains hippocampus and third ventricle. (HE, 6X)



1-2. Cerebrum, cat: The meninges and subjacent Virchow-Robin spaces are expanded by moderate numbers of macrophages, lymphocytes, and plasma cells, which extend into the adjacent neuroparenchyma. (HE, 122X)

National Veterinary Services Laboratory
RT-PCR - Positive for HPAI A(H5N1) virus,
clade 2.3.4.4b, genotype B3.13

Microscopic Description:

Throughout all examined sections of the cerebrum and within the brainstem, Virchow Robin spaces are mildly to moderately expanded by perivascular cuffs comprised of lymphocytes, histiocytes, and rare neutrophils ranging from 1-4 cell layers thick. The adjacent neuroparenchyma often contains increased numbers of scattered to small aggregates of glial cells admixed with low numbers of scattered lymphocytes, histiocytes, and rare neutrophils. Neuronal cell bodies within these regions and within the hippocampus are multifocally rimmed by increased numbers of glial cells, consistent with satellitosis. Neurons are occasionally shrunken, angular, and hypereosinophilic. The leptomeninges are mildly expanded by low to moderate numbers of lymphocytes and histiocytes, which multifocally infiltrate the surrounding cerebral grey matter. In one section, the adjacent grey matter contains numerous, multifocal small pools of perivascular hemorrhage rimmed by small amounts of karyorrhectic debris.

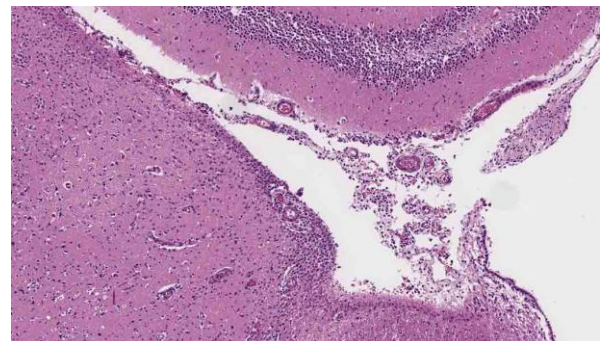
Contributor's Morphologic Diagnoses:

Cerebrum, brainstem, and meninges: Severe lymphohistiocytic meningoencephalitis with perivascular cuffing, multifocal hemorrhage, satellitosis, and neuronal necrosis

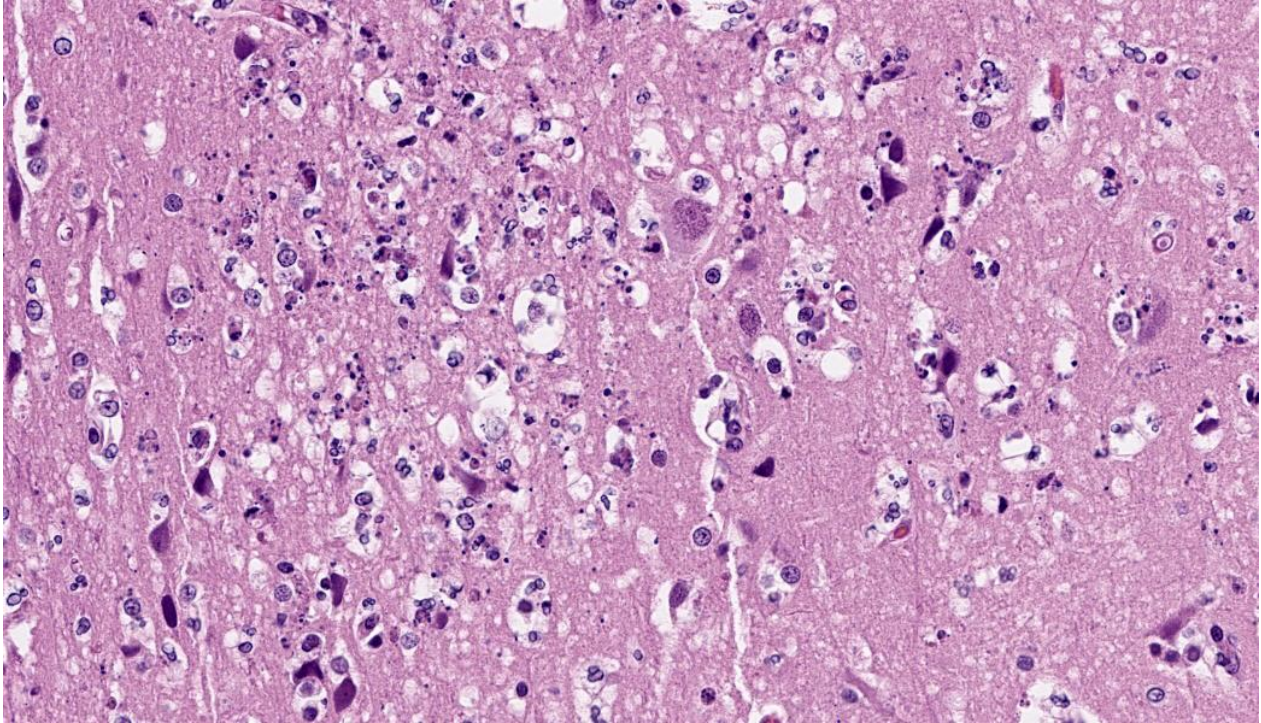
Contributor's Comment:

Over the last few decades, highly pathogenic avian influenza (HPAI) H5N1 has spread across the globe through the migration of numerous wild avian species.^{7,8} During this time, the H5 lineage has evolved within reservoir hosts, such as migratory waterfowl, via genetic drift and reassortment of viral RNA resulting in transformation of the virus into new strains which have led to devastating outbreaks within the poultry industry with concurrent spillover into an expansive array of mammalian hosts.^{7,8} Transmission from the reservoir hosts into spillover mammalian species can occur through direct transmission or adaptation of multiple viruses within intermediate hosts.^{7,8}

In general, the efficacy of influenza virus to target intermediate hosts and spillover species depends on the ability of the viral hemagglutinin molecules to bind to sialic acid residues on epithelial cell surface glycoproteins.⁸ These surface glycoproteins often vary between different tissues within the body and different species resulting in a wide array of



1-3. Cerebrum, cat: Similar changes are -present within the parenchyma adjacent to the third ventricle (HE, 171X)



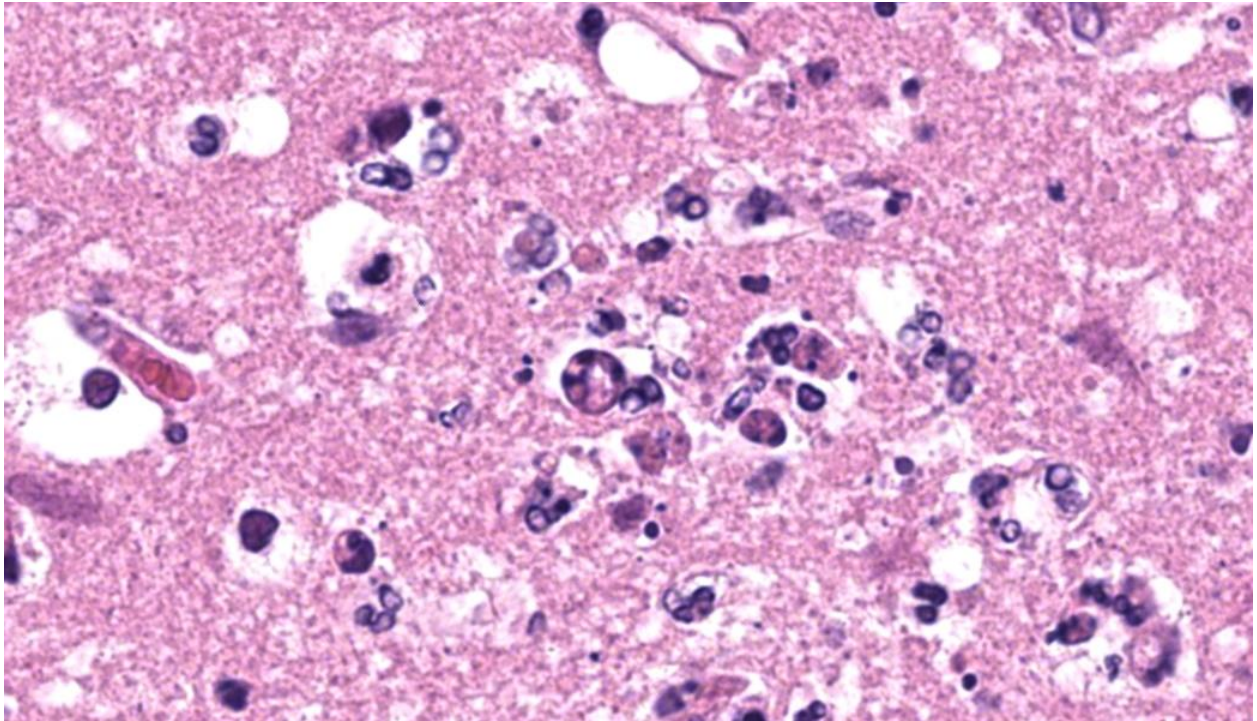
1-4. Cerebrum, cat: Inflammatory cells extend into the submeningeal gray matter, resulting in focal areas of necrosis and gliosis (HE, 657X)

clinical presentations dynamically impacting various animals as well as humans.⁸ While not completely elucidated, transmission of the virus from reservoir or intermediate hosts to other mammals is thought to mainly occur via predator/prey relationships by consumption of infected animals by carnivorous species.^{7,8} However, recent outbreaks have highlighted the importance of fomite transmission through viral shedding and environmental contamination.^{1,8}

Starting in March 2024, highly pathogenic avian influenza (H5N1) was identified in dairy cattle across the United States originating in Texas and spreading throughout multiple states, including Michigan.^{1,3,5} This strain of HPAI (H5N1, clade 2.3.4.4b) was detected in the United States in late 2021 to early 2022 and has been documented to affect multiple species, including cats.^{1,2,4,6,7} During the 2024 outbreak, HPAI was found in multiple dairy herds throughout Michigan with concurrent reports of feral or domesticated barn

cats afflicted by serious, fatal infections.¹ Previous HPAI outbreaks had similar reports with one of the first documented domestic feline HPAI H5N1 clade 2.3.4.4b infection occurring in France in December 2022 characterized by severe, fatal neurologic and respiratory signs which developed after close proximity to a duck farm with confirmed HPAI positive animals.^{2,6}

In this case, the affected cat was an indoor only cat with no history of direct contact with other affected animals or history of drinking unpasteurized milk. Further investigation revealed that one of the members of the family worked with a HPAI positive dairy farm. As such, infection of this animal is presumed to have occurred secondary to fomite transmission of the virus from clothes worn by this individual who worked closely with positive HPAI dairy cattle and reported exposure to unpasteurized milk to the face and clothing.⁶ Personal protective equipment was not uti-



1-5. Cerebrum, cat: High magnification of areas of gray matter necrosis with neuronal necrosis, satellitosis and infiltration by neutrophils and debris-laden macrophages admixed with cellular debris. (HE, 1750X)

lized, and the clothing was worn in the household prior to the development of neurologic signs and subsequent acute decline of the affected cat.⁶ Given the potential of fomite transmission, this case highlights the importance of utilizing personal protective equipment and strict potential post-exposure hygiene practices by veterinary professionals and people with careers where exposure to HPAI positive animals is possible.

Contributing Institution:

Michigan State University
Veterinary Diagnostic Laboratory
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Lansing, MI 48910
<https://cvm.msu.edu/vdl>

JPC Diagnoses:

Cerebrum: Meningoencephalitis, necrotizing and lymphohistiocytic, subacute, multifocal, marked with neuronal necrosis and gliosis

JPC Comment:

This week's moderator was Dr. Thomas Cerec, Clinical Professor of Anatomic Pathology in the Department of Biomedical Sciences and Pathobiology, at the Virginia-Maryland College of Veterinary Medicine, a popular moderator at the JPC who has abiding interest in infectious disease and microbial immunology.

Highly pathogenic avian influenza has been a frequent visitor to the WSC over the years with 15 entries including a range of poultry, waterfowl, and mammals including pigs, mustelids, and marine mammals, which demonstrates its increasing ability to a variety of avian and mammalian hosts.

A recent and interesting feature of the H5N1, clade 2.3.4.4b (and a potentially very worrisome one) is its ability to infect the mammary

gland of dairy cattle. This is not the first documented case of milk-associated virus transmission in ruminants, which has been previously identified in experimental infection of Powassan virus in goats, tick-borne encephalitis virus in cattle and goats, and Middle East Respiratory Coronavirus (MERS-CoV) has been detected in camel milk.⁹ Obviously however, the current ability of H5N1, clade 2.3.4.4b's ability to infect the mammary gland of domestic cattle and its ability to infect a wide range of additional hosts (including man) poses a significantly greater health risk than other viruses mentioned above.⁹ Pasteurization, however, has the ability to inactivate this virus.⁹

H5N1, clade 2.3.4.4b possesses not only the ability to infect the mammary gland but also can cause mastitis as well as produce heavy viral loads. Older multiparous animals appear to be more severely affected. Secretory epithelial cells in the bovine mammary gland express large amounts of the H5N1 viral entry receptor, α 2,3 sialic acid (while respiratory epithelium appears to express very few of these receptors, explaining why dairy cattle infected with this agent do not demonstrate significant respiratory disease.⁹ This finding also helps to explain why more severe clinical disease is seen in intramammary inoculated cattle over aerosol-infected animals. The U.S. Department of Agriculture has determined that milk from infected cows is the primary source of virus transmission to other dairy cattle, animals, and humans (Preliminary studies suggest that contaminated milking equipment, as well as infected air and water, may play a role in transmission between cows.⁹

Feline infection with HPAI is not a new occurrence, but previous infections have been associated with ingestion of infected poultry.

In 2004, a variety of domestic cats and large zoo felids in Thailand died following being fed poultry that had died following an HPAI infection.¹⁰ Lesions in natural and experimental infections are not confined to the nervous system, and mirror the widespread infection seen in a variety of avian and mammalian species. Degenerative vascular changes and thrombosis are seen in a range of organs, lymphoid depletion and necrosis of epithelial cells in the airways, pancreas, liver, and adrenal gland, and the kidney have been identified in affected felids.¹¹

Molecular analysis of H5N1 clade 2.3.4.4b infections in cats continue to yield clues to neurotropism of this virus in felids. Investigation of a 2024 outbreak which resulted in the deaths of ten cats in the same household with heavy viral loads in the brain demonstrated a number of unique viral mutations which may represent viral adaptation in this host species, including T143A in haemagglutinin, and two novel mutations in polymerase activity proteins (F314L, L342Q).¹²

Finally, another study demonstrates that brain is an excellent sample when testing for HPAI infection in cats, resulting in the lowest PCR cycle threshold values among sampled tissues. The authors suggest that random samples of Among these cases, brain was the best diagnostic sample, giving the lowest PCR Ct values when multiple sample types were tested. The authors propose that a random sampling of cerebral cortex is likely sufficient for testing given the abundant viral load seen in this organ in infected cats.¹³

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CASE II:

Signalment:

adult, female, water buffalo (*Bubalus bubalis*)

History:

One of three water buffaloes found dead peracutely in a herd of 14 animals was submitted for necropsy.

Gross Pathology:

Tongue: Severe, multifocal to coalescing erosions and ulcerations affecting approximately 30% of the dorsal lingual mucosa.

Rumen: Severe, multifocal to coalescing erosions and ulcerations, predominantly affecting the ruminal pillars.

Heart: Multifocal, pale, streaky discoloration of the myocardium.

Other: Generalized congestion, cyanosis, and pulmonary edema.

Laboratory Results:

PCR:

BTV, BVDV, BHV-1, OvHV-2 negative

FMDV positive

Microscopic Description:

At low magnification, multifocal areas of pallor correspond to the grossly observed streaks within the myocardium. At higher magnification, there is multifocal degeneration and necrosis of cardiomyocytes, characterized by hypereosinophilia, loss of cross striations, and occasional fragmentation. Associated with necrotic myocardial cells, there is mild to moderate inflammatory infiltration composed mainly of histiocytes and fewer lymphocytes. The myocardial interstitium is



2-1. Heart, water buffalo: Two sections of heart are submitted for examination. At subgross magnification, there are linear bands of pallor within the myocardium. (HE, 10X).

mildly expanded by edema. Immunohistochemistry reveals intracytoplasmic labeling of cardiomyocytes for FMDV antigen.

Contributor's Morphologic Diagnoses:

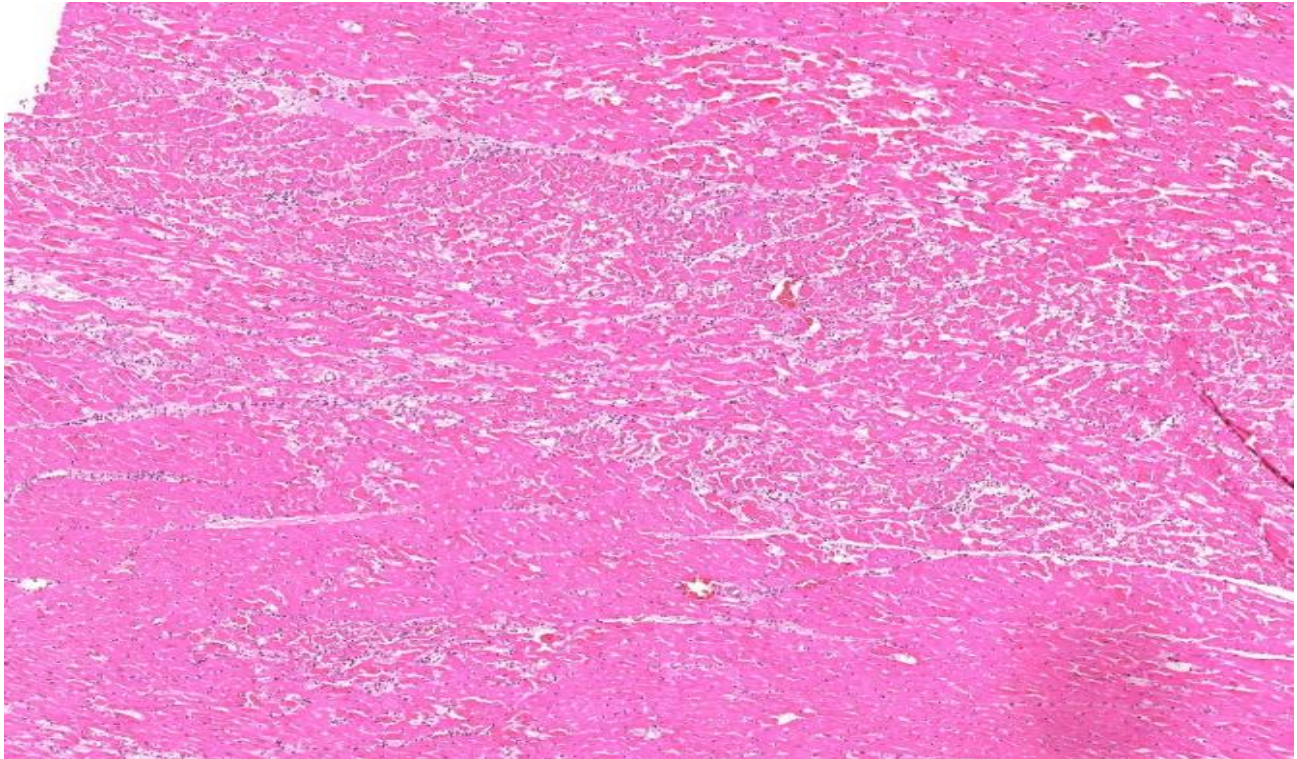
Myocardial degeneration and necrosis, acute, multifocal, with mild lymphohistiocytic myocarditis

Contributor's Comment:

The foot-and-mouth disease virus (FMDV), a member of the genus *Aphthovirus* within the family *Picornaviridae*, causes a highly contagious disease of cloven-hoofed animals and solipeds. Seven different serotypes are recognized: O, A, C, Asia 1, SAT1, SAT2, and SAT3.¹

Transmission most commonly occurs through direct contact between infected and susceptible animals, but can also occur via contaminated materials, including feed.² Clinical signs vary depending on age and species; however, characteristic findings include fever and the formation of vesicles (aphthae) that progress to erosions and ulcerations on the oral mucosa, tongue, hooves, and udder.³

In addition to epithelial lesions, FMDV can cause degeneration and necrosis of cardiomyocytes due to viral replication within these cells. This may result in the characteristic



2-2. Heart, water buffalo: There are linear areas of myofiber degeneration and necrosis within the myocardium. (HE, 86X).

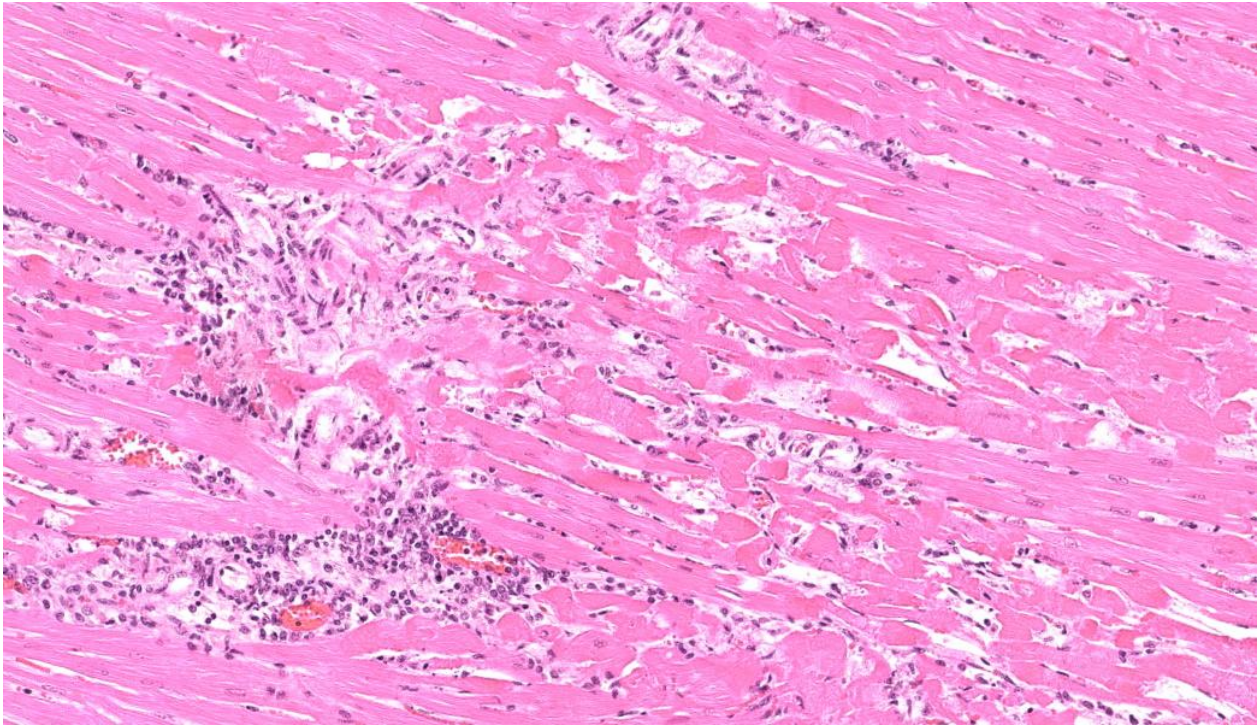
“tiger heart” appearance, caused by streaks of pale myocardial fibers, as observed grossly in this case. Myocardial involvement is most common in young animals but may also occur in adults and can lead to peracute death without preceding clinical signs. The pathogenesis of FMDV-associated myotropism is not fully understood but is thought to involve a combination of viral and host factors, including age-related susceptibility.³ For in vivo diagnosis, measurement of serum cardiac troponin I levels can be useful, as this biomarker is specific for myocardial cell injury.⁴ Histopathologically, myocardial lesions vary with age and disease progression. In young animals (e.g., calves, piglets, and neonatal lambs), cardiomyocyte degeneration and necrosis often occur with minimal inflammatory response. In contrast, older animals more commonly develop myocarditis

characterized by lymphohistiocytic inflammatory infiltrates, as seen in this case.⁵

Differential diagnoses for myocardial degeneration and necrosis in ruminants include nutritional myodegeneration (vitamin E/selenium deficiency), toxic cardiomyopathies, and infectious causes. Bacterial agents (e.g., *Clostridium* spp., *Histophilus somni*) and parasitic infections (e.g., *Sarcocystis* spp., *Neospora caninum*) are more common, whereas viral myocarditis is comparatively rare. In addition to FMDV, encephalomyocarditis virus should also be considered.⁵

Contributing Institution:

Department of Experimental Facilities and Biosafety,
Friedrich-Loeffler-Institute
<https://www.fli.de/en/institutes/departments/experimental-animal-facilities-and-biorisk-management-atb/>



2-3. Heart, water buffalo: Higher magnification of an area of myofiber degeneration and necrosis. There are moderate numbers of lymphocytes and histiocytes in areas of the mildly edematous interstitium. (HE, 282X).

JPC Diagnoses:

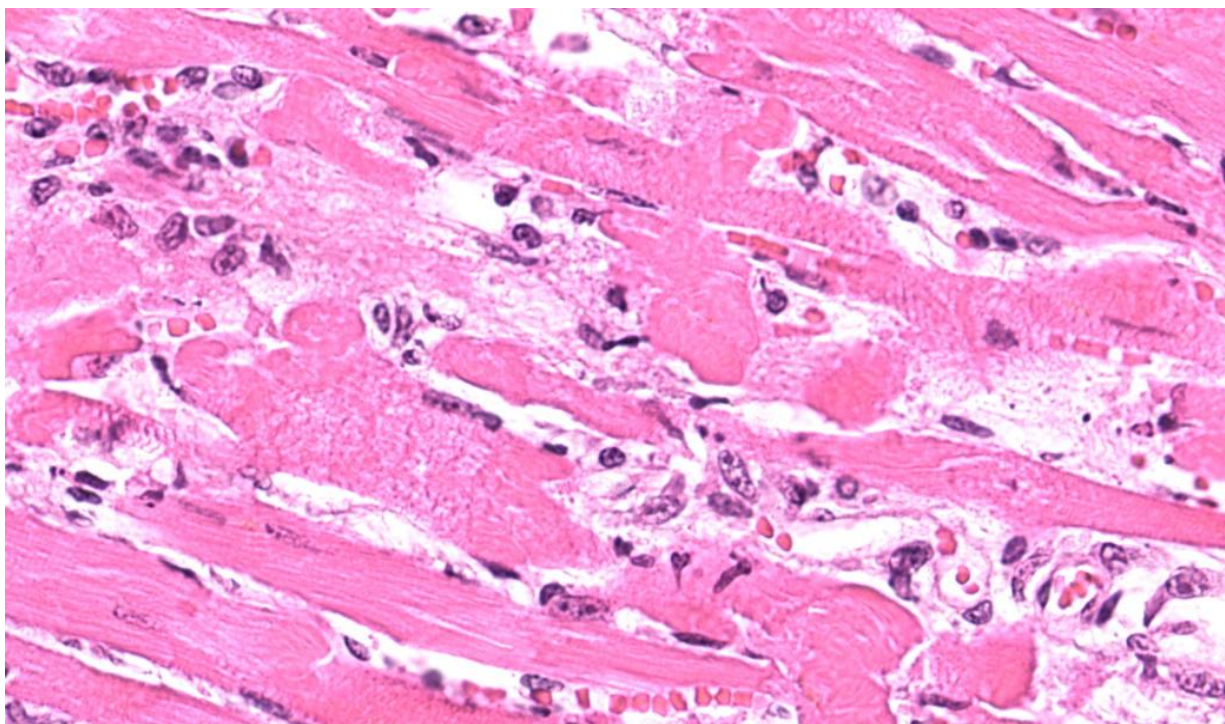
Heart: Cardiomyocyte degeneration and necrosis, subacute, multifocal to coalescing, with mild multifocal lymphohistiocytic myocarditis.

JPC Comment:

The trifecta of foot and mouth disease, a water buffalo, and our longstanding contributor, the Fredrich Loeffler Institute may seem like an unusual combination, so a bit of explanation is in order. In 2024, three individuals in a herd of 14 water buffalo in Brandenburg, Germany died within 48 hours.⁶ The owner transported one carcass to the state diagnostic lab, which loss of approximately 1/3 of the lingual epithelium, necrotizing myocarditis and pulmonary edema were identified, and tissues were positive for FMD RNA by real-

time PCR (and were corroborated by the FRG National Reference Laboratory. Immediately following these results, the remainder of the herd and 261 susceptible animals (245 pigs, 13 sheep and 3 goats) in three nearby farms and 61 additional susceptible animals (3 cattle, 39 sheep, 19 goats) at a farm that had received hay from the infected farm were culled and tested. The European Union Animal Disease Information System (EUADIS) and the World Animal Health Information System (WAHIS) were notified and the World Organization for Animal Health (WOAH) suspended Germany's FMD-free status.⁶

The initial tested buffalo had no anti-FMDV antibodies but the remainder of the animals in the herd did. Nine of the remaining buffaloes demonstrated oral lesions, and two had pedal lesions. FMDV antigen and viral genome was



2-4. Heart, water buffalo: Higher magnification of an area of myofiber degeneration and necrosis with myofiber pallor, vacuolation, formation of contraction bands, pyknosis of satellite nuclei, and fragmentation (HE, 1054X).

identified in the myocardium of the initially necropsied individual. Based on the coding region of the viral capsid protein VP1 (633 nucleotides), the virus isolated from this buffalo was identified as FMDV serotype O, topotype ME-SA and lineage 115 SA-2018. The route of introduction of this outbreak was not identified, and modeling suggested minimal farm connectivity and a low potential for additional transmission. Response to an O/PanAsia-2 vaccine strain was promising, but ultimate the decision not to vaccinate was made by the authorities.⁶

Water buffaloes are an important part of agriculture in much of the world, especially in tropical and subtropical countries. Buffalo farming has gained popularity in these areas, as in addition to being an effective draft animal, they also provide high-quality red meat and milk. In Bangladesh, farmed buffalo exceed 1.5 million.⁷

While the literature on FMD infection in water buffaloes is somewhat sparse (but growing, as their popularity increases), the disease is like that seen in cattle. While not associated with high mortality in adults, the lesions result in significant loss of feed conversion (not to mention the economic impact of depopulation.) Lesions in both species are characterized by extensive loss of mucosal epithelium within the gastrointestinal tract and in areas of friction such as the interdigital space and coronary band. In both species, the main lesion in calves less than 6 months of age is necrotizing myocarditis (as seen in this case), which, in buffaloes may range up to 50% in calves less than 1 year and up to 100% in suckling animals.⁸ Other risk factors for FMD in buffaloes include co-grazing with cattle, exposure to bushy environments, inadequate deworming and vaccination practices, and insufficient isolation facilities.⁸

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CASE III:

Signalment:

Male Holstein fetus aborted at 234 days of gestation, *Bos taurus*, bovine.

History:

An adult, female, second-gestation Holstein cow aborted a male fetus at 234 days of gestation.

Gross Pathology:

The fetus was in a good state of postmortem preservation, with a complete hair coat. No external abnormalities were noted. There was mild subcutaneous edema in the ventral neck, diffuse petechiae in the thymus, and paintbrush hemorrhages in the serosa/adventitia of the umbilical arteries. The abdominal, thoracic, and pericardial cavities contained a moderate amount of reddish serous fluid. In both kidneys there were numerous, discrete, pinpoint red foci with a generalized distribution alternating with areas of pallor visible from the capsular surface, which on cut section corresponded with enhanced cortical and



3-1. Kidney, aborted calf. There are numerous discrete pinpoint red foci disseminated throughout the renal cortex that are visible through the capsular surface. (Photo courtesy of: Viruses. 2022; 14:2042. doi: 10.3390/v14092042, reprinted with permission.)



3-2. Liver, aborted calf. Cut section of the hepatic parenchyma showing an enhanced reticular pattern characterized by numerous pinpoint pale grayish foci and interconnected linear streaks alternating with reddish-orange areas of hepatic parenchyma ("nutmeg liver"). (Photo courtesy of: Viruses. 2022; 14:2042. doi: 10.3390/v14092042, reprinted with permission.)

medullary rays in the renal parenchyma. The liver had multifocally extensive areas of roughness and pale whitish discoloration visible from the diaphragmatic capsular surface; the underlying parenchyma had increased consistency and an enhanced reticular pattern, characterized by numerous pinpoint pale grayish foci, and indistinctly bordered interconnected linear areas ~1 mm wide alternating with reddish-orange areas of parenchyma.

Laboratory Results:

None before the pathologic examination was conducted (see the "Contributor's Comment" section below for results of laboratory tests conducted postmortem).

Microscopic Description:

Kidney: with a multifocal disseminated distribution the cortical tubules are variably ectatic (luminal dilation), frequently contain hypereosinophilic and karyorrhectic (necrotic) cell debris in their lumina and are lined by attenuated epithelium. Multifocally

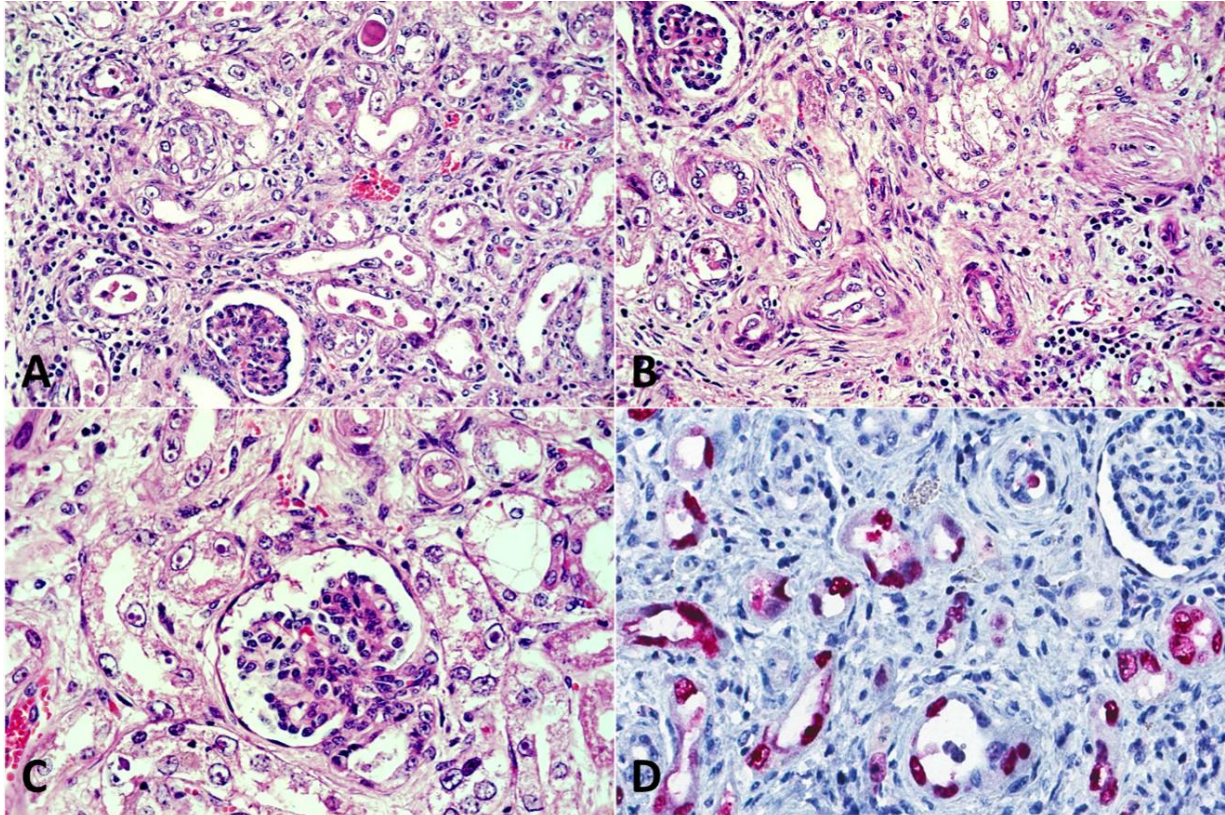
tubular epithelial cells are either swollen and have vesicular nuclei (tumefaction) frequently containing one or more round or pleomorphic magenta viral inclusion bodies, surrounded by a clear halo (chromatin margination), or are shrunken with angular cell borders, hypereosinophilic cytoplasm and have pyknotic nuclei or karyorrhectic debris (necrosis). In affected areas, the interstitium is multifocally infiltrated by inflammatory cells, predominantly lymphocytes and histiocytes, with some plasma cells and occasional neutrophils, and/or expanded by spindle cells with an elongated nuclei (fibroblasts) embedded in a collagenous eosinophilic fibrillar extracellular matrix (fibrosis/fibroplasia).

Contributor's Morphologic Diagnoses:

Kidney: nephritis, tubulointerstitial, lymphocytic, and histiocytic, with intranuclear epithelial viral inclusion bodies, cortical tubular epithelial cell necrosis, and interstitial fibrosis, multifocally extensive, severe, bovine.

Contributor's Comment:

In this case, bovine polyomavirus 1 (BoPyV-1, *Epsilonpolyomavirus bovis*) was detected by qPCR, isolated from the kidney, and fully sequenced, while other viruses that can cause intranuclear inclusions (such as herpesviruses and adenoviruses) were ruled out. In addition, abundant polyomavirus large T antigen (LT Ag) was demonstrated intralesionally by immunohistochemistry in cortical tubular epithelial cells (Figure 3D), and large numbers of viral particles with typical polyomaviral morphology were found in the nuclei of renal tubular epithelial cells by transmission electron microscopy (Figure 4). Further details on the diagnostic investigation conducted in this case have been recently published.⁸



3-3. Kidney, aborted calf. A- Cortical tubules are variably ectatic, contain necrotic cellular debris and are lined by either attenuated epithelium, or epithelial cells with markedly swollen vesicular nuclei, occasionally containing one or several magenta intranuclear inclusion bodies. The cortical interstitium is infiltrated by inflammatory cells, predominantly lymphocytes and histiocytes. Hematoxylin and eosin (HE) stain. **B-** Similar histologic lesions as described in A but there is also marked interstitial fibrosis. HE stain. **C-** Numerous cortical tubular epithelial cells have markedly swollen nuclei containing one or several magenta intranuclear inclusion bodies surrounded by a clear halo (arrows), indicating margination of the chromatin. HE stain. **D-** Immunoreactivity to polyomavirus LT antigen is demonstrated by red chromogen precipitate largely in the nucleus of cortical tubular epithelial cells. Immunohistochemistry (immunoalkaline phosphatase technique) with a monoclonal primary antibody against SV-40 polyomavirus LT antigen, hematoxylin counterstain. (*Photo courtesy of: Viruses. 2022; 14:2042. doi: 10.3390/v14092042, reprinted with permission.*)

The pathological findings in the kidneys of the fetus were consistent with an active polyomavirus infection, with lytic replication of the virus, production of abundant intranuclear inclusion bodies composed of dense arrays of assembled virions, and LT Ag expression in the nuclei of renal tubular epithelial cells. In addition, severe degeneration (i.e., swelling, tumefaction, attenuation) and necrosis of renal tubular epithelial cells were indicative of a cytopathic viral effect, along

with a prominent inflammatory response to the infection, and interstitial fibrosis suggestive of chronic active renal damage at the time of abortion.

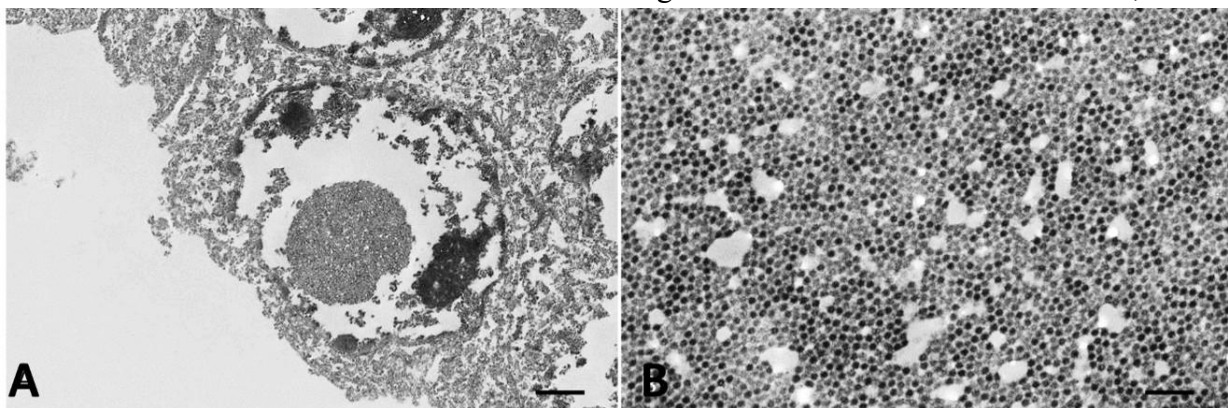
Polyomaviruses are a diverse group of non-enveloped viruses with a small, circular, double-stranded DNA genome found in a wide variety of mammalian (including humans), avian, fish, amphibian, reptile, and inverte-

brate species.¹² According to the International Committee on Taxonomy of Viruses (ICTV, <https://talk.ictvonline.org/>, accessed on June 20, 2023), the *Polyomaviridae* family contained 8 genera named Alpha-, Beta-, Delta-, Epsilon-, Eta-, Gamma-, Theta- and Zeta-polyomavirus, although many strains and species were awaiting genus assignation. While some polyomaviruses can cause acute disease and even death due to productive (lytic) replication in their hosts, most of the polyomaviruses infecting mammals establish persistent subclinical infections in healthy individuals, resulting in clinical disease only after reactivation of the infection in immunosuppressed hosts.¹¹

Most information on the biology and medical relevance of polyomaviruses has been generated in studies of primate and murine polyomaviruses; limited information is available from other mammalian polyomaviruses in their natural hosts. Prototype diseases caused by human polyomaviruses include “polyomavirus-associated nephropathy” (PyVAN) caused by Human polyomavirus-1 (BK polyomavirus –BKPyV–, *Betapolyomavirus hominis*) and to a lesser extent Human poly-

omavirus-2 (JC polyomavirus –JCPyV–, *Betapolyomavirus secuohominis*) in kidney transplant recipients; and “progressive multifocal leukoencephalopathy” (PML) resulting from replication of JCPyV in oligodendrocytes in patients with acquired immunodeficiency syndrome or other immunomodulatory conditions.¹² Polyomaviruses have also been associated with cancer in humans and animals. For example, Human polyomavirus-5 (Merkel cell polyomavirus –MCPyV–, *Alphapolyomavirus quintihominis*) causes Merkel cell (neuroendocrine) carcinoma of the skin,⁶ while Procyon lotor polyomavirus-1 (syn. Raccoon polyomavirus-1 –RacPyV-1–, *Alphapolyomavirus procyonis*) has been associated with tumors of the olfactory tract and brain in raccoons.^{1,5,9}

The polyomaviruses currently known as bovine polyomavirus (BoPyV) were initially discovered in uninoculated cultures of kidney cell lines of stump-tailed macaques, rhesus monkeys, and cynomolgus macaques, and were referred to as Stump-Tailed Macaque Virus (STMV),¹⁸ cynomolgus kidney strain (CK-strain) of STMV,²⁰ and Fetal Rhesus Kidney Virus (FRKV).¹⁵ Because of their growth in uninoculated cell cultures, it was



3-4. Kidney, aborted calf. A- The nucleus of a tubular epithelial cell contains a dense array of viral particles forming a round inclusion (7190 $\times$, bar = 2 μ m). **B-** Higher magnification of A showing the array of viral particles (70,000 $\times$, bar = 200 nm). (Photo courtesy of: Viruses. 2022; 14:2042. doi: 10.3390/v14092042, reprinted with permission.)

initially thought that they were endogenous viruses of non-human primates. However, it has since been determined that they were polyomaviruses of bovine origin contaminating the bovine fetal serum used to supplement cell growth media.¹⁴ The first polyomavirus isolated in a bovine kidney cell line was obtained from a healthy newborn calf,² and was designated Wokalup Research Station Virus (WRSV). It was suggested shortly after their discovery that STMV, FRKV, and WRSV were all isolates of an identical BoPyV,¹³ which was later supported by viral whole-genome sequencing.¹⁹

Species of polyomaviruses known to infect live cattle to date include *Bos taurus* polyomavirus-1 (syn. Bovine polyomavirus-1 – BoPyV-1–, *Epsilonpolyomavirus bovis*), and *Bos taurus* polyomavirus-2 (syn. Bovine polyomavirus-2 –BoPyV-2–, awaiting genus assignment). A third species named Bovine polyomavirus-3 (BoPyV-3, awaiting genus assignment) was originally detected in ground beef samples collected at a supermarket in the USA.¹⁶ To the best of our knowledge, BoPyV-1 and BoPyV-3 have never been causally associated with pathology or disease, while BoPyV-2 has been recently proposed as a probable cause of non-suppurative encephalitis in cattle.¹⁰

Although the pathogenic mechanisms by which most polyomaviruses induce tissue damage are not yet fully understood, five patterns of polyomavirus-induced pathology have been proposed.⁴ These include: 1- cytopathic polyomavirus pathology pattern, 2- cytopathic-inflammatory pathology pattern, 3- immune-reconstitution inflammatory syndrome, 4- autoimmune polyomavirus pathology pattern, and 5- oncogenic polyomavirus pathology pattern. The cytopathic polyomavirus pathology pattern is characterized by

uncontrolled viral replication in the infected cells without significant inflammation; PML caused by JCPyV replication in the oligodendrocytes is the prototype disease for this pattern. The cytopathic-inflammatory pathology pattern is characterized by high-level virus replication with cytopathic lysis of infected cells, along with necrosis and a significant inflammatory response. PyVAN in kidney allografts is the prototype disease for this pattern.⁴ The lesions in the kidneys of the aborted fetus described herein best fit with this latter pattern of polyomavirus-induced pathology.

Histologically, PyVAN in humans is characterized by varying degrees of multifocal random interstitial nephritis, tubulitis, cytopathic changes and basophilic intranuclear inclusions in renal epithelial cells, interstitial fibrosis, and tubulo-interstitial atrophy, depending on the stage of disease progression.¹⁷ Interestingly, these same lesions were observed in the kidneys of the aborted bovine fetus infected with BoPyV-1.⁸

Immunohistochemistry using antibodies against Simian Virus 40 (SV-40, *Macaca mulatta* polyomavirus 1, *Betapolyomavirus macacae*) LT Ag, as in this case, has been shown to cross-react with other polyomaviruses, such as BKPyV and JCPyV, in cases of PyVAN in humans.³ In concert with this, the antibody also cross-reacted with BoPyV-1, indicating that this test can be used for viral identification in formalin-fixed paraffin-embedded bovine tissues. This is not unexpected, considering that LT antigen has domains that are conserved among the polyomaviruses.¹²

Generally, occurrence and progression of diseases caused by polyomaviruses in mammals

seem to be largely dependent on host immunosuppression.^{11,12} Pregnant females can experience modulation in their immune system making them more prone to pathogenic infections.⁷ While a real-time PCR performed on serum of the cow indicated that she was viremic at the time of the abortion,⁸ whether she or the aborted fetus were immunosuppressed was unknown. There was no evidence of lymphoid depletion on histologic examination of lymphoid tissues including thymus, spleen, and lymph nodes in the aborted fetus. Bovine viral diarrhea virus, a common immunosuppressive virus of cattle, was ruled out by reverse transcriptase qPCR in the fetal tissues.

This case suggests that BoPyV-1 is pathogenic to the bovine fetus and, thus, a probable cause of abortion in cattle.⁸ Pathogenicity seems to involve cytopathic viral effects with tissue damage including necrosis, inflammation, and fibrosis, with lesions resembling PyVAN in humans. Factors of the virus and host that would determine lesion and disease development, as well as the epidemiology and transmission routes, need further investigation.

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JPC Diagnoses:

Kidney: Tubular degeneration, necrosis, and regeneration, chronic, diffuse, moderate, with cellular and granular casts, interstitial nephritis and fibrosis, and tubular epithelial intranuclear viral inclusions.

JPC Comment:

The contributor has provided an outstandingly thorough review of this entity. Polyomaviruses are also not uncommon visitors to the Wednesday Slide Conference with 18 cases dating back to 1994 (and likely quite a few before that).

Half of these cases are the various manifestations of primate polyomavirus (of which SV-40 is the prototypical NHP polyomavirus. This polyomavirus manifests in the brain, kidney, and in one case in the WSC Archive (WSC 1997, Conference 14, Case 1), in the lung (as well as in the kidney), representing well-known syndromes associated with polyomavirus infection in NHPs. In each of these cases, the affected non-human primate was immunosuppressed following experimental infection with similar lentivirus. In immunosuppressed non-human primates, polyomavirus infection results in Simian virus 40 (SV40) is an endemic polyomavirus in captive and free-ranging macaques. Infection does not cause lesions in immunocompetent animals, but immune suppression, such as SIV infection or immunosuppressive drug regimens, allows reactivation of latent infections or primary infections to cause disease.

There are two forms of nervous system disease in immunosuppressed macaques associated with polyomavirus infection. Progressive multifocal leukoencephalopathy (PML) results in demyelinating disease due to infection of oligodendroglia and is thought to occur in animals with underlying SV40 infection that subsequently are immunosuppressed.^{22, 23} A second nervous system manifestation of SV40 infection (in younger animals, but not necessarily immunosuppressed animals, is meningoencephalitis (ME), which is characterized by inflammatory infiltrates

within the cortical grey matter and leptomeningeal edema.^{22,23} Renal infections in immunosuppressed macaques provide a model mirroring polyomavirus-associated renal transplant rejections in humans, and the pneumonia seen in immunosuppressed macaques mirrors a severe, rapidly fatal pneumonia seen in humans receiving hematopoietic stem cell transplants.

Several distinct syndromes associated with polyomavirus in birds have been seen in the WSC over the years. Budgerigar fledgling disease is a disease of young hand-reared psittacines which results in necrosis and intranuclear inclusions within tubular epithelium somewhat reminiscent of today's case in an aborted calf. Affected budgerigars that do not rapidly succumb to this disease may develop a range of feather abnormalities known as "French molt". Viral-associated hepatic necrosis is seen in affected birds as well. A single case of goose hemorrhagic polyomavirus (one of the four known avian polyomaviruses) appeared in the WSC in 2002 ([Conference 7, Case 4](#)), and is the only one of the avian polyomaviruses in which intranuclear viral inclusions are not seen.²⁴

One final polyomavirus that has appeared several times in the WSC over the years is a unique polyomavirus infecting hamsters. This polyomavirus possesses the ability to infect both lymphocytes and undifferentiated keratinocytes. In young naïve animals, epizootic polyomavirus infection causes lymphoma, resulting in mortality between 4 and 30 weeks. In older animals, enzootic polyomavirus infection of undifferentiated keratinocytes results in the generation of multiple benign follicular tumors similar to trichoepitheliomas.²⁵

Note: Images 3-1 through 3-4, and their respective captions were recently published⁸ in the journal *Viruses* (please see citation below), in an open access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 International license (CC BY 4.0, <https://creativecommons.org/licenses/by/4.0/>). The contents of the article can be shared, copied, and distributed freely in any medium or format.

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CASE IV:

Signalment:

Five, 23-day-old, Commercial broilers, *Gallus gallus*

History:

4 day illness reported by the overseeing veterinarian.

Gross Pathology:

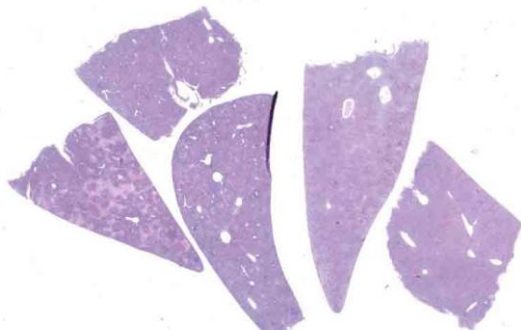
Field necropsy, no gross lesions provided.

Laboratory Results:

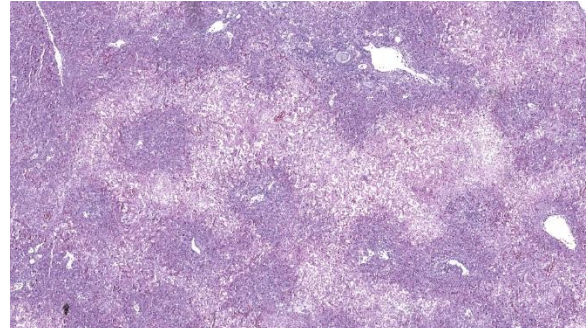
None available

Microscopic Description:

Liver: Multiple and often coalescing foci of necrosis (severity varies between sections) are randomly present throughout the section. The surrounding and intervening hepatocytes are often vacuolated (degenerate). Multifocal



4-1. Liver, chicken: Sections of liver from multiple birds are submitted for examination. Even at this low magnification, retiform areas of hepatocellular necrosis are evident. (HE, 10x).



4-2. Liver, chicken: Higher magnification of area of coagulative necrosis within the hepatocellular parenchyma. (HE, 87x).

cally, hepatocytes contain round, amphophilic to basophilic smudgy intranuclear inclusion bodies that fill and expand the nucleus. Varying infiltrates of lymphocytes, plasma cells, and macrophages frequently expand the parenchyma. Medium to large numbers of lymphocytes and plasma cells as well as varying numbers of hematopoietic precursors expand portal and centrilobular connective tissues.

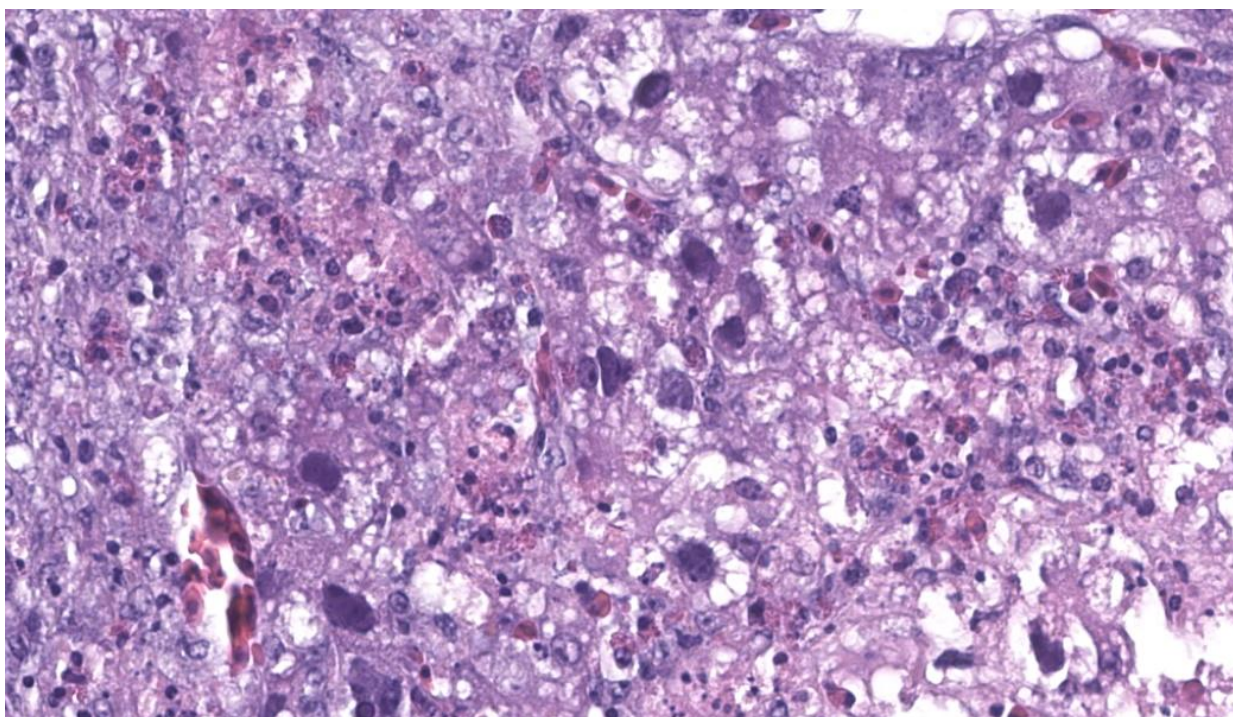
Contributor's Morphologic Diagnoses:

Liver: Multifocal to coalescing, variably severe, hepatic necrosis with intranuclear, basophilic inclusion bodies and marked lymphoplasmacytic to granulomatous inflammation

Contributor's Comment:

Fowl adenoviruses are a ubiquitous class of viruses that can cause a wide range of disease in poultry and other bird species including inclusion body hepatitis (most commonly serotype 8), gizzard erosion (most commonly serotype 1), and hydropericardium syndrome (most commonly serotype 4).⁴

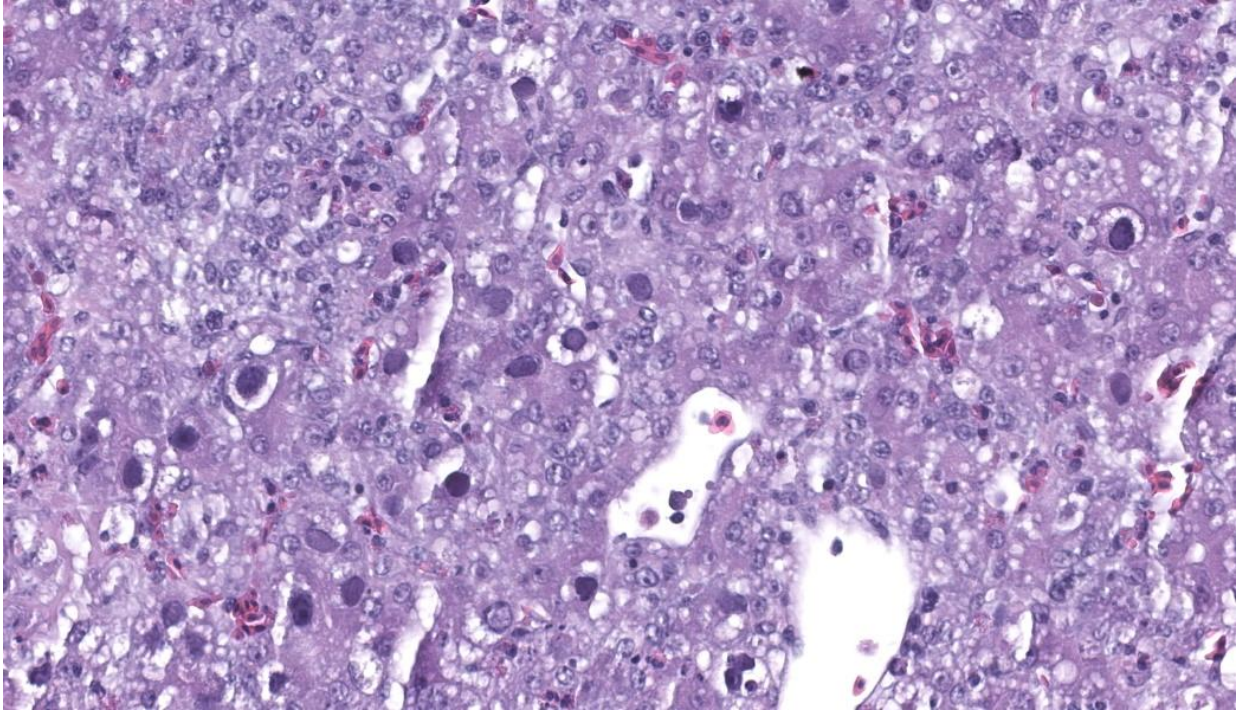
Recent updates in taxonomic classification have identified the etiology of inclusion body hepatitis as *Aviadenovirus hepatitidis* (FAdV-E), serotypes 8a and 8b (FAdV-8a, -8b).² The virus is transmitted horizontally by



4-3. Liver, chicken: In this field with single-cell and larger areas of necrosis (bottom right), there are numerous karyomegalic basophilic intranuclear adenoviral inclusions. (HE, 1077X)

feco-oral routes and vertically from infected breeder hens shedding virus in eggs.⁶ Infected birds can shed virus for up to 6 weeks and it is fairly resistant once in the environment.⁶ Though any age or breed of bird can potentially be infected and develop clinical disease, young broilers 5-8 weeks old are most common.^{2-4,6} This is likely due to waning of maternal immunity. It is worth noting that birds infected vertically tend to develop disease at a slightly younger age. Once infected, the most common clinical sign is a sudden, dramatic increase in mortality, with loss of >30% of the flock possible. Grossly, animals with IBH often have pale, enlarged livers, but may also have mottled and enlarged kidneys and spleens.¹ Atrophy of the thymus and bursa are variable and may be influenced by the presence of concurrent, immunosuppressive viral infections such as infectious bursal disease or chick anemia virus.⁴ Occasionally, multifocal petechiae are

present in pelvic limb skeletal muscle. Histologically, there is acute necrosis in the liver with varying amounts of inflammation. Characteristic adenovirus inclusions are almost diagnostic in these cases. These are large, amphophilic to basophilic, intranuclear inclusions that often enlarge the nuclear contour enhancing their prominence. Less commonly, inclusions will occasionally be slightly eosinophilic with a clear halo resembling herpesvirus inclusions.¹ Though the liver is consistently affected, viral inclusions and/or necrosis can also be variably observed in the pancreas, kidneys, gizzard, and intestines of infected birds.³ Diagnosis is typically reached via a combination of compatible gross and histologic findings and molecular diagnostics. There is no widely accepted treatment although supportive care may be warranted in clinically ill birds if they do not succumb to the infection. Prevention of clin-



4-4. Liver, chicken: Viral inclusions are widespread, even in areas of the liver without significant necrosis (yet). (HE, 1077X)

ical infection through vaccination and management of stress and concurrent disease are the goals of management practices.^{2,6} Vaccination of parent stock with autogenous vaccines developed via isolation of the challenge subtype virus from progeny has been shown to be effective in prevention of vertical transmission.⁵ It may also improve prevention of horizontal infection as well.

Contributing Institution:

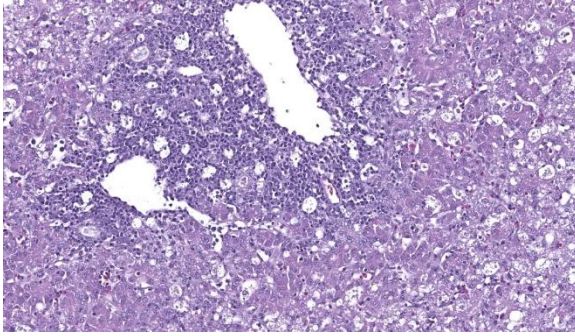
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VA-MD College of Vet Med,
Virginia Polytechnic Institute and State University
<https://vetmed.vt.edu/>

JPC Diagnoses:

Liver: Hepatitis, necrotizing, subacute, multifocal to coalescing, random, moderate with numerous intranuclear viral inclusions.

JPC Comment:

The genus *Aviadenovirus*, formerly classified as group I avian adenovirus, includes pathogens associated with inclusion body hepatitis, quail bronchitis, and hydropericardium syndrome. *Siadenovirus*, formerly group II avian adenovirus, includes turkey hemorrhagic enteritis virus (THEV), marble spleen disease (MSD) virus in pheasants, and the chicken “splenomegaly virus,” which causes a disease resembling THEV and MSD. This genus is genetically distinguished by a sialidase-encoding gene and also includes a frog adenovirus. *Atadenovirus*, formerly group III avian adenovirus, includes the egg drop syndrome virus, which causes reduced egg production and thin-shelled eggs in its natural hosts, ducks and geese. The genus also includes viruses that infect mammals and reptiles.⁷



4-5. Liver, chicken: A cellular combination of EMH, lymphocytes and plasma cells extend into the adjacent parenchyma. (HE, 387X)

As noted by the contributor, IBH virus primarily targets hepatocytes, but pancreatic lesions are reported as well. Infection tends to occur in 3–7-week-old broiler chickens (although it has been reported in birds as young as 7 days and as old as 20 weeks) resulting in up to 30% mortality. It often occurs as a secondary infection in immunodeficient birds with other diseases, predominantly infectious bursal disease (birnavirus, serotype 1) and chicken infectious anemia (circovirus). Outbreaks of IBH with similar gross and histological lesions have also been reported in columbiformes, psittacines and raptors.^{1,8,9} The recently identified falcon adenovirus, which is distantly related to fowl adenovirus types 1 and 4.

Infection with fowl adenovirus type 4 is the cause of hydropericardium syndrome virus (Angara disease), which is found in the Middle East¹⁰ as well as South America; particularly severe manifestations are also associated with immunosuppression. Lesions include pericardial effusion, pulmonary edema, hepatomegaly and renomegaly; mortality can range from 20-80%; and affected broilers are usually 3-5 weeks old.⁸ In this condition, the pericardium is filled with a straw-colored fluid and is accompanied by hemorrhage in

the heart and pulmonary edema. Gross and histologic lesions typical of IBH are seen in this disease, and necrosis and inclusions may also be seen in the gizzard, pancreas, duodenum, proventriculus, cecum and kidney.¹⁰

In a retrospective study of IBH in chickens, Stoute *et al.*¹¹ identifies other histologic findings in almost 350 cases of IBH, including the presence of gizzard erosions (associated with inclusions within glandular epithelium) and glomerulopathy (characterized by inclusions in renal endothelium, expansion of the glomerulus with amorphous eosinophilic material, tubular dilation with hyaline cast formation, and urate tophe).¹¹ Lymphoid tissue were diffusely depleted and the bone marrow was aplastic (with likely contributions by immunosuppressive birna- and circoviruses as mentioned above.)

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