



WEDNESDAY SLIDE CONFERENCE 2017-2018

Conference 15

17 January 2018

CASE I: S16-1796 (JPC 4101316).

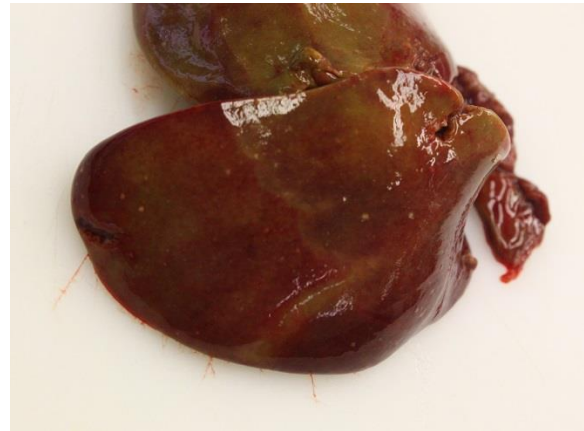
Signalment: 21-week-old, male, Eagle owl, *Bubo bubo*, avian.

History: The animal's general condition was reduced (sunken eyes, bristling of the feathers, anorexia) and got constantly worse over 3 days. The animal died subsequently despite treatment with glucose and activated charcoal. The animal originated from a falconry, was kept in an aviary with the possibility of free flight and was mostly fed on chicken and pigeons.

Gross Pathology: The animal was in moderate body condition. The liver had a light brown to beige color. On the surface, randomly distributed on all lobes, small (<1 mm in diameter), sharply demarcated, whitish-yellowish foci were found. On the mucosa of the small intestine, randomly distributed, round, 2 mm in diameter, well demarcated, whitish-yellowish foci were detected. The spleen was considerably swollen and showed a dark red to light violet color. Round, whitish-yellowish foci were also detected on the surface of the spleen.

Laboratory results:

Bacteriological examination of liver (culture): Negative.



Liver, owl. There are multiple well-demarcated areas of necrosis scattered through the liver parenchyma. (Photo courtesy of: Institute of Veterinary Pathology Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich, Fax number +41 44 635 89 34, www.vetpathology.uzh.ch)

Parasitological examination of feces:
Negative.

Microscopic Description: Liver: Approximately 70% of the liver had multifocal to coalescing randomly distributed foci of coagulative necrosis, characterized by hypereosinophilic hepatocytes with karyopyknosis and karyorrhexis. The inflammatory response surrounding foci of necrosis was minimal and consisted of a few macrophages. Intra-nuclear eosinophilic inclusion bodies and

chromatin margination occurred in hepatocytes bordering the necrotic areas.

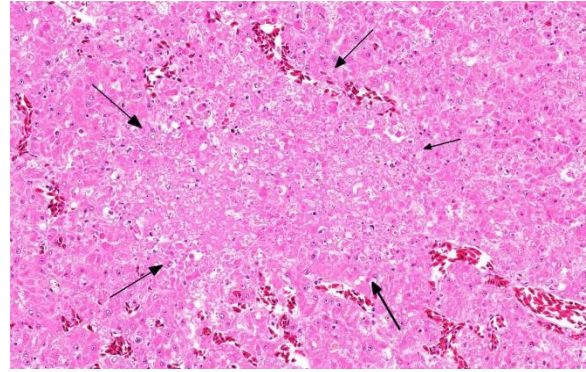
Contributor’s Morphologic Diagnosis:

Liver: Hepatitis, multifocal, acute, necrotizing, severe with intranuclear eosinophilic inclusion bodies within hepatocytes.

Contributor’s Etiologic Diagnosis:
Columbid herpesvirus 1

Contributor’s Comment: Gross and histologic lesions in this owl were highly suspicious of an infection with herpesvirus and this was confirmed by the detection of the characteristic intranuclear inclusion bodies in hepatocytes bordering the necrotic foci. In addition to necrotizing hepatitis, necrotizing splenitis and necrotizing enteritis was seen in this animal. The owl has been kept in an aviary and fed on chicken and pigeons, the latter being the most likely source of infection; pigeons are often subclinically infected with columbid Herpesvirus 1 that can cause disease in owls.⁸

In 1932 in North America herpesvirus was first reported in owls and subsequently in prairie falcons (*Falco mexicanus*), American kestrels (*Falco sparverius*) and the peregrine falcon (*Falco peregrinus*).⁸ Originally herpesvirus isolates from falcons and owls have been considered as distinct viruses, namely falconid herpesvirus 1 (FHV-1) and strigid herpesvirus 1 (StHV-1). However, serologic studies showed cross-reactivity among FHV-1, StHV-1 and columbid herpesvirus-1 (CoHV-1) as well as that CoHV-1 and FHV-1 are indistinguishable and finally PCR studies confirmed that inclusion body hepatitis in falcons and owls is caused by columbid herpesvirus-1.³



Liver, owl. Areas of largely coagulative necrosis are scattered throughout the section (delimited by arrows). The lack of inflammatory cells suggests an acute lesion. (HE, 168X)

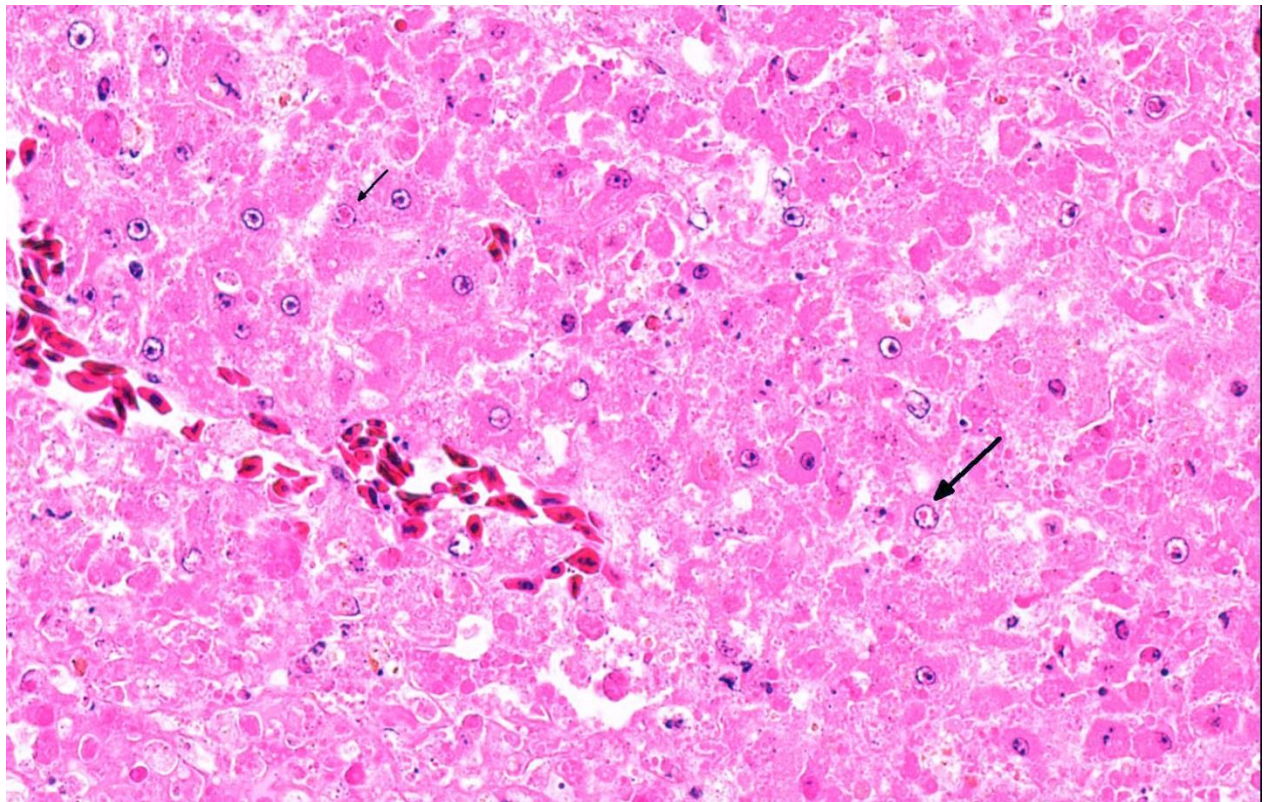
In owls, falcons, and eagles, the disease is known as hepatosplenitis. The disease usually has an acute to subacute course. Clinical signs are nonspecific, including weakness, anorexia and depression.⁷ Characteristic gross lesions in falcons are small white hepatic and splenic foci representing necrosis and similar foci in the bone marrow of falcons.³ Histologically, areas of coagulative necrosis are detectable in the spleen, liver and bone marrow, usually with only little associated inflammation.³ Intranuclear eosinophilic inclusion bodies within hepatocytes or macrophages bordering the necrotic areas can frequently be detected.³ Lesions caused by CoHV-1 are not restricted to the liver and spleen; often the small intestine and the kidney are also involved.⁶ The progression of the disease is associated with rapid virus multiplication and organ dysfunction. Most reported cases of disease in falcons and owls involve prior documented or possible ingestion of pigeons or by direct contact with the infected bird.^{4,8}

In pigeons, Hhrpesvirus-induced disease was first described in Rock Pigeons (*Columbia livia*) and was called “inclusion body disease” or “inclusion body hepatitis”.³ Herpesvirus is prevalent in the pigeon population and has little to no effect on the

health of this species. Pigeons, especially those kept in captivity, are common subclinical carriers of the virus. However, disease occurs in squabs aged ten to sixteen weeks. Clinical signs such as depression, anorexia, conjunctivitis, oral and pharyngeal ulceration, dyspnea, and diarrhea with a duration of a few hours to as long as one week are reported, but not always present. Histological lesions include hepatic and splenic necrosis as reported in owls and falcons. In addition upper respiratory tract inflammation with ulceration and upper gastrointestinal tract inflammation with ulceration is described. Epithelial and parenchymal cells bordering the necrotic areas contain eosinophilic intranuclear inclusions.^{3,4}

The disease is transmitted to squabs by chronically infected male and female

breeding pigeons when feeding regurgitated crop milk during the first weeks of life of the squabs.⁵ Contact during courtship, preening and mutual feeding of adult pairs during mating does not result in virus transmission. Ingested virus replicates in the oropharynx region, followed by short-term viremia and virus multiplication in all internal organs. Squabs show severe epithelial lesions in the pharynx, esophagus and crop. Infection by pigeon herpesvirus only rarely results in clinically overt forms of disease in adults and adult pigeons usually do not present any clinical signs except depression, anorexia or conjunctivitis.⁵ Exact data on the prevalence of herpesvirus disease in pigeons is not available. Numerous reports provide evidence for the presence of the pigeon herpesvirus in all European countries and many pigeon lofts. Pigeon herpesvirus has



Liver, owl. At the edge of the areas of necrosis, degenerating hepatocytes often contain a single eosinophilic intranuclear inclusions. (HE, 400X)

been detected in all breeds of domestic pigeons (*Columba livia f. domestica*), feral pigeons, and other members of the family Columbidae.⁵

JPC Diagnosis: Liver: Hepatitis, necrotizing, random, multifocal to coalescing, moderate with intranuclear eosinophilic viral inclusion bodies, Eagle owl (*Bubo bubo*), avian.

Conference Comment: Hepatosplenitis caused by herpesvirus in owls (OHV) and falcons (FHV) are genetically similar and are both pathogenic for owls, ring-necked doves (*Streptopelia* sp.) and kestrels (Eurasian and American).² In a comprehensive review of OHV, Drs. Burtscher and Sibalin reviewed the wide spectrum of hosts affected and identified the tawny owl and barn owl as being resistant.¹ Herpesviral disease in birds of prey is often Peracute, resulting in rapid fatalities. However, subclinical cases do occur and are characterized by lethargy, anorexia, diarrhea, and a progressive leukopenia. Gross lesions include hepatomegaly and splenomegaly with pharyngeal and intestinal lesions in owls. Microscopically, there is hepatic necrosis with prominent intranuclear inclusion bodies most prominent at the edge of the necrotic tissue.²

The virus is often spread through infected pigeons, as in this case, and is a risk to both captive and free-living birds of prey around the world. Diagnosis is usually based on gross lesions and identification of the characteristic eosinophilic intranuclear inclusion bodies microscopically which are pathognomonic for this disease.⁹ There is no successful treatment for viral hepatitis and most often infected birds are culled to prevent spread of the disease. In comparison to psittacine herpesviruses, falcon and owl

herpesviruses are more resistant to chemical disinfectants.²

The main differential for liver and intestinal lesions in raptors with prominent intranuclear inclusion bodies would be adenovirus. However, most of these animals are subclinically infected and diagnosis is often made at necropsy.²

Conference participants noted that there was some slide variation with mixed colonies of bacteria in areas of necrosis on some slides (presumed postmortem overgrowth) and intranuclear inclusion bodies in bile duct epithelial cells.

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CASE II: E 6940/16 (JPC 4100856).

Signalment: 4-week-old, female, Chilean flamingo, *Phoenicopterus chilensis*, avian.

History: A 4-week-old, female flamingo from the zoo showed a multinodular, ulcerated, wart-like proliferation of approximately 5 x 4 x 3 cm extension in the skin at the right tibiotarsal joint. The proliferated tissue was surgically resected, fixed in 10% neutral buffered formalin and submitted for histological examination.

Gross Pathology: The submitted tissue was partially ulcerated and had a tan color. On cut surface it appeared multilobulated.

Laboratory results:

Formalin-fixed paraffin-embedded tissue (FFPE) was used for molecular sequencing of the gene encoding the 4b-protein. Phylogenetic analysis revealed a sequence homology of 99.9% with the ATCC strain of canarypox (genus: avipoxvirus).

Microscopic Description: Glaborous skin: The epidermis of the featherless skin is irregularly proliferated and severely thickened with increased layers of spinosum cells (acanthosis). There are multifocal superficial or complete losses of the epidermis associated with extravascular erythrocytes and few heterophilic granulocytes. Underneath the stratum corneum there are multifocal accumulations of partly degenerated heterophilic granulocytes, erythrocytes, proteinaceous fluid and bacteria. Particularly, cells of the spinosum layer display severe diffuse hypertrophy with intracellular edema (hydropic degeneration). Eosinophilic inclusion bodies up to 15 µm in diameter are present in the cytoplasm (Bollinger's inclusion bodies). Within the dermis, there is a diffuse mild to moderate infiltration of heterophilic granulocytes and few macrophages. Furthermore, numerous blood vessels are markedly extended and filled with red blood cells. Multifocally there are moderate accumulations of extravascular erythrocytes (hemorrhages) and eosinophilic, fibrillary material (fibrin).

Contributor's Morphologic Diagnosis:

Skin: Dermatitis, erosive and ulcerative, heterophilic, acute, diffuse, severe with epidermal hyperplasia, pustules, hydropic degeneration of keratinocytes and cytoplasmic, eosinophilic inclusion bodies (Bollinger's inclusion bodies) consistent with poxvirus infection.

Contributor's Comment: The morphological findings are consistent with a

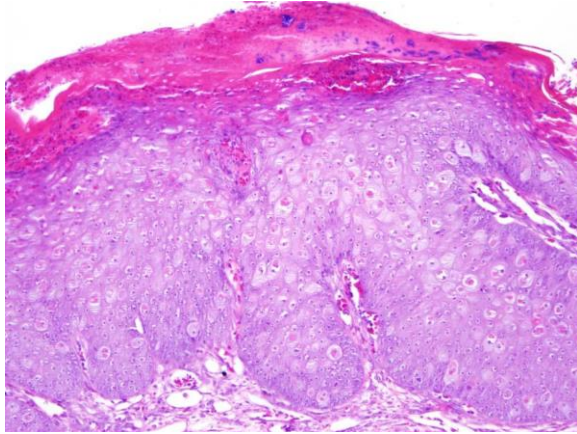


Glabrous skin, flamingo. There is a 5 x 4 x 3 cm multinodular, ulcerated, wart-like cutaneous mass at the right tibiotarsal joint. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie>)

poxvirus infection that was confirmed by transmission electron microscopy. Molecular analysis revealed a canarypox strain of the genus avipoxvirus (APV). The histologic key lesions include epidermal hyperplasia and hydropic degeneration of keratinocytes with large, cytoplasmic, eosinophilic inclusion bodies (Bollinger's inclusion bodies). Using the pop-off technique,¹¹ transmission electron microscopy (TEM) revealed biconcave brick-shaped virions measuring 250 x 320 nm. Virus particles exhibited, depending of the sectioning plane, a biconcave core, two lateral bodies and an envelope consistent with avipox virions.¹⁵

Macroscopically, an exophytic ulcerated multinodular proliferation was present on the featherless skin at the tibiotarsal joint. This wart-like lesion represents the

proliferative or cutaneous form of an APV infection ("dry pox").⁸ It is characterized by nodular proliferations on featherless skin such as legs, feet, eyelids and base of the beak. Scars may be visible after recovery and healing. Another manifestation of APV infections is termed diphtheritic/diphtheroid or "wet" form that is characterized by proliferative and fibrino-necrotic lesions of the mucous membranes, predominantly of the tongue, pharynx and larynx.^{4,8,20} Birds may also show both forms. The mortality rate of the diphtheritic form is reported to be higher compared to the cutaneous form. However, secondary bacterial infections may significantly increase the mortality rate in the cutaneous form.²⁰ Rarely, a septicemic form develops that is characterized by acute onset of ruffled plumage, somnolence, cyanosis and anorexia. This form may cause mortality rates of up to 99% and is seen



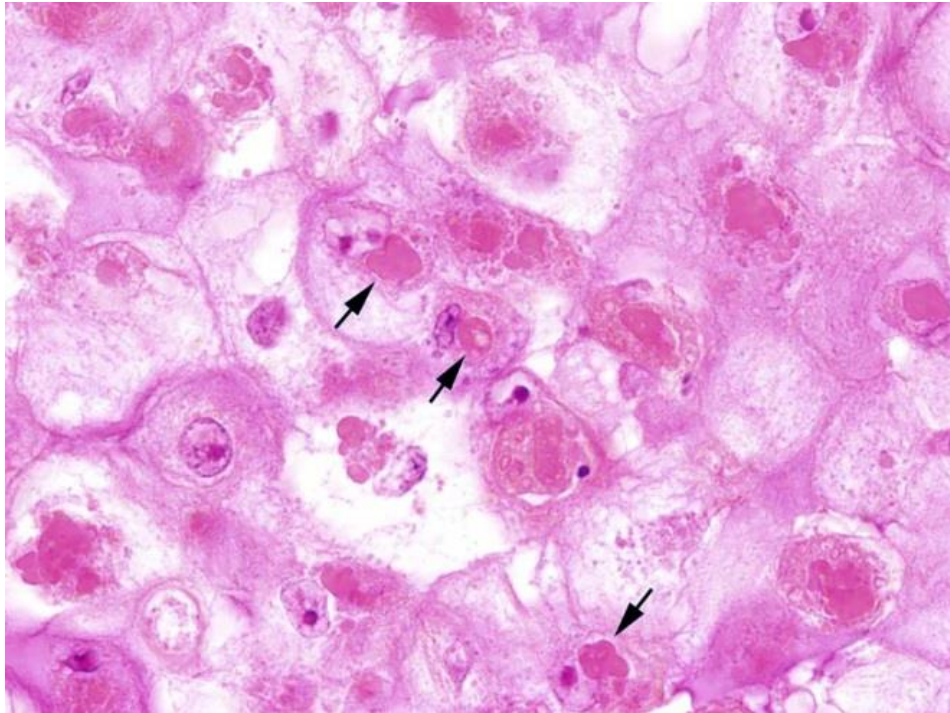
Glabrous skin, flamingo. . There is irregular, frond-like proliferation of the featherless skin with a markedly thickened stratum spinosum . Numerous keratinocytes contain large round intracytoplasmic viral inclusions. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie>)

predominantly in canaries and canary-finch crosses.⁸

Avian poxviruses belong to the genus *Avipoxvirus* which is a member of the subfamily *Chordopoxvirinae* with the family of *Poxviridae*. Avian poxviruses are large, brick-shaped, enveloped viruses with a double-stranded DNA. In infected cells, they replicate in the cytoplasm.^{10,19} Transmission occurs through latently infected birds and biting arthropods. In addition, direct transmission of the virus may be facilitated by small traumatic injuries caused by territorial behavior. Furthermore, virus may be transmitted through aerosols via mucous membranes of the eyes or the upper respiratory and digestive tracts.^{4,8,10,12} Mosquitos may retain infectious virus in the salivary glands for 2 to 8 weeks.⁸ Dry scabs can harbor the virus for many months.¹⁵

Birds of all ages are susceptible, however, mostly young individuals are affected. The incubation period varies from 7 to 14 days.¹⁴ The virus is usually named by the species in which it was originally isolated. Most investigations about mortality and morbidity of APV infections are based on single APV isolates, which make it difficult to find general information on pathogenicity of particular APV isolates in different species. For example, canaries are highly susceptible to canary poxviruses but they are resistant to pigeon pox, turkey pox and fowl pox.¹⁹ Nevertheless, APV can also cross species barriers and may infect taxonomically different species.¹⁰ Avian poxvirus has a worldwide distribution and infection is described in over 232 avian species in 23 orders.² Disease can arise in domestic, pet and wild birds of many different species.¹⁹

Avipoxvirus infections have been described in various flamingo species in different countries of the world.^{10,13,16,20} American flamingos (*Phoeniconais ruber*) were infected in the USA and Portugal,^{10,13} Lesser flamingos (*Phoenicopterus minor*) in South Africa,¹⁶ and Greater flamingos (*Phoenicopterus roseus*) in Japan.¹⁶ In the presented case, a Chilean flamingo (*Phoenicopterus chilensis*) was affected. In all published cases, infection occurred in young individuals up to 4.5 months of age. They all suffered from the cutaneous form of avian poxvirus infection.^{8,11,16,20} In Portugal, Japan and the USA, single animals were affected,^{4,10,13} whereas in South Africa 30% of the fledgling flamingos displayed the cutaneous form.²⁰



Glabrous skin, flamingo. Keratinocytes of the stratum spinosum are swollen as a result of intracytoplasmic edema (ballooning degeneration) and often contain one or more irregularly round intracytoplasmic viral inclusions (Bollinger bodies) (arrows). (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie>) (HE, 400X)

Identification and differentiation of various Avipoxvirus species is mainly based on sequencing of the 4b core polypeptide. This gene is composed of 1971 nucleotides and encodes a protein that has a molecular weight of 75.2 kDa.^{1,18} For the isolation of Avipoxvirus, the chorioallantoic membrane (CAM) of specific-pathogen-free (SPF) chicken embryos is inoculated. Within this culture system the virus forms type A cytoplasmic inclusions.^{2,10,13}

As morphological differential diagnoses neoplastic proliferations, granulomatous inflammation as well as exuberant granulation tissue have to be considered.

JPC Diagnosis: Skin: Dermatitis, necrotizing and proliferative, focally extensive, severe, with ballooning

degeneration, and intracytoplasmic eosinophilic viral inclusion bodies (Bollinger bodies), Chilean flamingo (*Phoenicopterus chilensis*), avian.

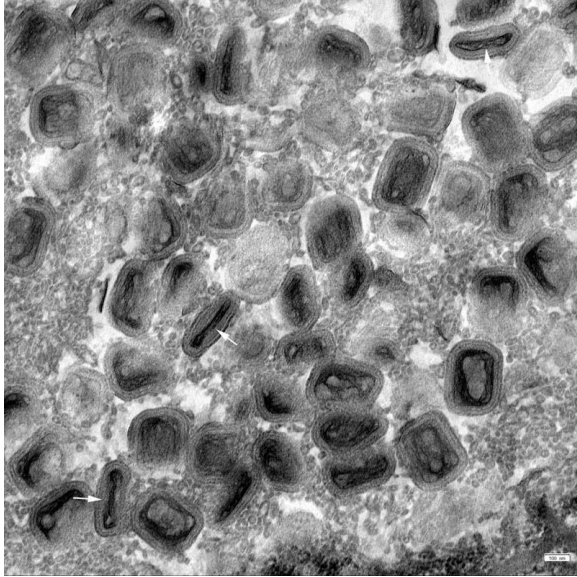
Conference

Comment: The term fowlpox was initially used to describe poxvirus infections of all birds, but as the number of species affected grew, it became used specifically for the disease in chickens.³

Avianpox is an old disease that was previously thought to be related to human small pox and chicken pox. While

this disease does not affect the human population, it does affect numerous avian species that we know of (chickens, turkeys, pigeons, canaries, psittacines, and wild birds) but perhaps all bird species are susceptible.¹⁷

The first USDA license issued for a poultry product was for the fowlpox vaccine in 1918.⁵ To this day, the pox vaccine is the primary method of disease control and prevention with initial vaccination of birds at 4 weeks of age or at any age if necessary. The fowl pox vaccine is currently being used as a vector for recombinant vaccines due to its efficacy and prevalence.¹⁷ The characteristic inclusion bodies of poxviruses (described above) represent the site of DNA synthesis and packing of the infectious virus particles. Avianpox viruses contain



Glabrous skin, flamingo. Ultrastructurally, infective keratinocytes contain numerous biconcave brick-shaped virions measuring 250 x 320 nm. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie>)

numerous genes for DNA replication, repair, and processing, as well as a specific enzyme (CPD photolyase) that repairs UV-induced DNA damage using visible light as a source of energy. This may help explain the virus' environmental durability. Poxviruses encode proteins that affect host cells such as vaccinia virus growth factor (VGF) which

stimulates proliferation of keratinocytes using epidermal growth factor receptors (EGFRs). Still other proteins inhibit complement mediated cell lysis and the host inflammatory response. All of these factors function to not only provide the virus a safe environment to replicate in, but also provide fertile soil for secondary bacterial infections.³

Gross differentials for cutaneous (dry) pox include mite infections and bacterial pododermatitis. *Cnemidoptes mutans* ("scaly leg mite") lives primarily in unfeathered skin and causes thick, hyperkeratotic shanks with white, scaly crusts, and *Cnemidoptes gallinae* ("depluming mites") lives in basal feather shafts and causes breakage or complete loss of feathers and intense irritation.⁶ Finally, bacterial pododermatitis ("bumblefoot") most commonly caused by *Staphylococcus aureus* results in purulent abscesses on the plantar surface of the foot due to penetrating wounds.⁷

Conference participants noted variable serocellular crust formation in some sections with prominent colonies of superficial bacteria admixed with hemorrhage.

Table 1: Select genera of the family Poxviridae.^{9,12}

Genus	Virus/Disease	Major Hosts
<i>Orthopoxvirus</i>	Vaccinia virus Buffalopox/Rabbitpox virus*	Numerous: cattle, buffalo, swine, rabbits
	Cowpox*	Rodents (reservoir), cattle, cats, elephants, rhinos
	Camelpox	Camels
	Ectromelia (Mousepox)	Mice, voles
	Monkeypox*	NHPs, squirrels, anteaters
<i>Capripoxvirus</i>	Goatpox	Goats, sheep

	Sheeppox	Sheep, goats
	Lumpy skin disease virus	Cattle, cape buffalo
<i>Suispoxvirus</i>	Swinepox virus	Swine (vector= <i>Hematopinus suis</i>)
<i>Leporipoxvirus</i>	Myxoma virus	Rabbits (<i>Oryctolagus</i> & <i>Sylvilagus</i> spp.)
	Rabbit fibroma virus, Hare fibroma virus	Rabbits
	Squirrel fibroma virus	Grey and red squirrels
<i>Avipoxvirus</i>	Fowlpox, canarypox, quailpox, etc	Chickens, turkeys, peacocks, etc.
<i>Parapoxvirus</i>	Caprine parapoxvirus (Orf; contagious ecthyma)*	Sheep, goats
	Bovine parapox (bovine papular stomatitis virus)*	Cattle
	Pseudocowpox*	Cattle
	Sealpox*	Seals
	Parapoxvirus of red deer	Red deer
<i>Molluscipoxvirus</i>	Molluscum contagiosum virus*	NHPs, birds, dogs, kangaroos, equids
<i>Yatapoxvirus</i>	Yabapox virus & tanapoxvirus*	NHPs
Unclassified	Squirrel poxvirus, fish (carp edema), horsepox	

*zoonotic

Contributing Institution:

<http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie>

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CASE III: LJ84 (JPC 4101222).

Signalment: 1-year-old, male, Indian rhesus macaque, *Macaca mulatta*, primate.

History: Received bivalent pneumococcal/salmonella vaccination a year prior and 100 TCID50 of SIVmac251 intravenously five months prior to submission. Initially presented with soft stool and distended abdomen 2 weeks prior to being discovered recumbent with nystagmus and head tilt.



Abdomen, rhesus macaque A section of spinal cord is presented for examination. There is bilateral spongiosis of the dorsal aspects of the ventral funiculi. (Photo courtesy of: Tulane National Primate Research Center, www2.tulane.edu/tmprc/)

Gross Pathology: The abdomen contains 20-30 ml of blood-tinged fluid. The pancreas is enlarged (5.5 x 8 x 3 cm) and hemorrhagic (Fig 1). There is edema of the capsule of the left kidney and the serosa of the duodenum. Formed feces noted in the colon.

Laboratory results:

	RBC	Hgb	Hct	WBC
	PMN	Eo	Bas	Mon
	Plt	Retic		Lym
- 5 months	5.18	12.1	39.1	8.55
	41.6	1.6	0.2	7.1
	404K	0		49.5
Presented	3.77	8.0	26.2	29.4
	90.0	0	6.0	4.0
	138K	10.3		

Blood chemistry is unremarkable.

Microscopic Description: Pancreas: The capsule of the pancreas is edematous and diffusely hemorrhagic and infiltrated with numerous neutrophils and small numbers of eosinophils and macrophages. Fibrin tags containing neutrophils are attached to the peritoneal surface. Large zones of the acinar pancreas are necrotic with multifocal hemorrhage and infiltration of variable

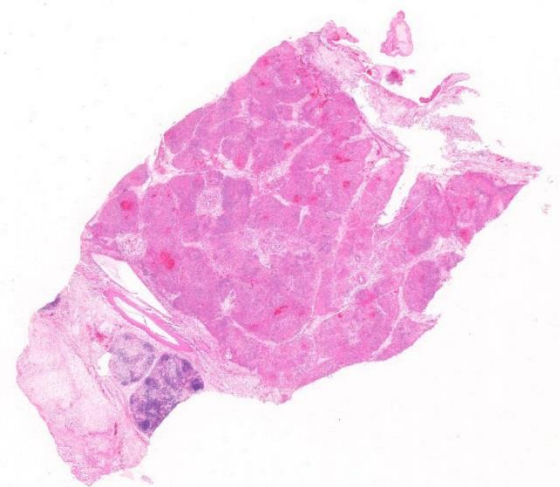
numbers of neutrophils and small numbers of macrophages. Some blood vessels contain fibrin thrombi; vascular walls contain segmental foci of degeneration with PMN infiltration or complete necrosis. Surviving epithelial cells are swollen and many contain slightly enlarged nuclei with marginated chromatin and glassy basophilic intranuclear inclusions. IFA staining with anti-adenoviral monoclonal antibody confirms the presence of adenovirus in epithelial cell nuclei and cytoplasm. Stroma is edematous.

Contributor’s Morphologic Diagnosis:

Pancreas: Pancreatitis, acute hemorrhagic, necrotizing, Adenovirus with fibrinous peritonitis and necrotizing vasculitis.

Contributor’s Comment:

Acute pancreatitis in humans is most often (80%) associated with biliary tract disease and alcoholism.⁵ Clinical signs include epigastric abdominal pain (80-95%), nausea and vomiting (40-80%), and abdominal distension (21-46%) with some displaying fever, jaundice, ascites, and pleural

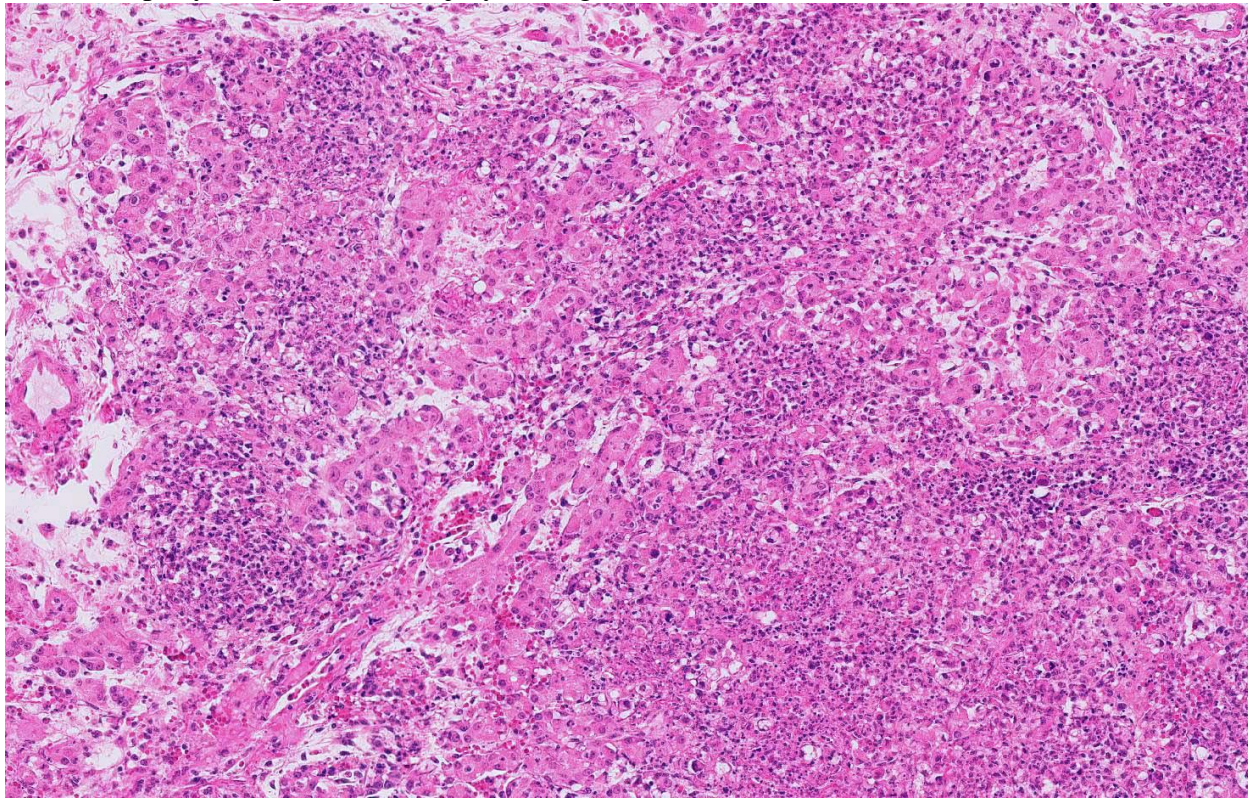


Pancreas, rhesus macaque. A section of pancreas, and adjacent mesentery and lymph node is presented. The pancreas, while maintaining its overall lobular architecture, lacks the characteristic basophilia of a healthy pancreas. (HE, 5X)

effusion.² In most instances, gallstones and/or inflammation reduces outflow of digestive proenzymes, allows back-diffusion of these secretions across the pancreatic ducts, and activation of the proenzymes.⁵ High ethanol concentrations can induce spasm or edema of the sphincter of Oddi, as well as induce production of secretin from the small intestine triggering more pancreatic secretions.⁷ Protease inhibitors like alpha-1-antitrypsin, alpha-2-macroglobulin, c-1-esterase inhibitor, and pancreatic secretory trypsin inhibitor in body fluids and tissues protect against activation of nascent proenzymes stored and transported in membrane bound granules. However, protection is incomplete since, in the presence of calcium, trypsin bound to inhibitor still has some tryptic activity that activates other proenzymes.⁷ Damage to acinar cell membranes, ducts and blood vessels rapidly compounds the injury adding

hemorrhage and anoxia.

Other causes of acute pancreatitis include drugs, hyperlipidemia, hypercalcemia, viral infections (mumps, coxsackievirus, hepatitis B, CMV, varicella, herpes simplex, adenovirus, HIV), bacterial and fungal infections (Mycoplasma, Legionella, Leptospira, Salmonella, Aspergillus, Toxoplasma, Cryptosporidium), vascular diseases, pregnancy and ascariasis.⁶ Drugs like the thiazide diuretics induce hypercalcemia; estrogens and HIV protease inhibitors induce hypertriglyceridemia with production of toxic free fatty acids, vascular damage and thrombosis.⁶ Blood and bone marrow transplantation have been linked to acute pancreatitis by a variety of mechanisms including drug toxicity, graft-versus-host-disease, and adenoviral infection.³

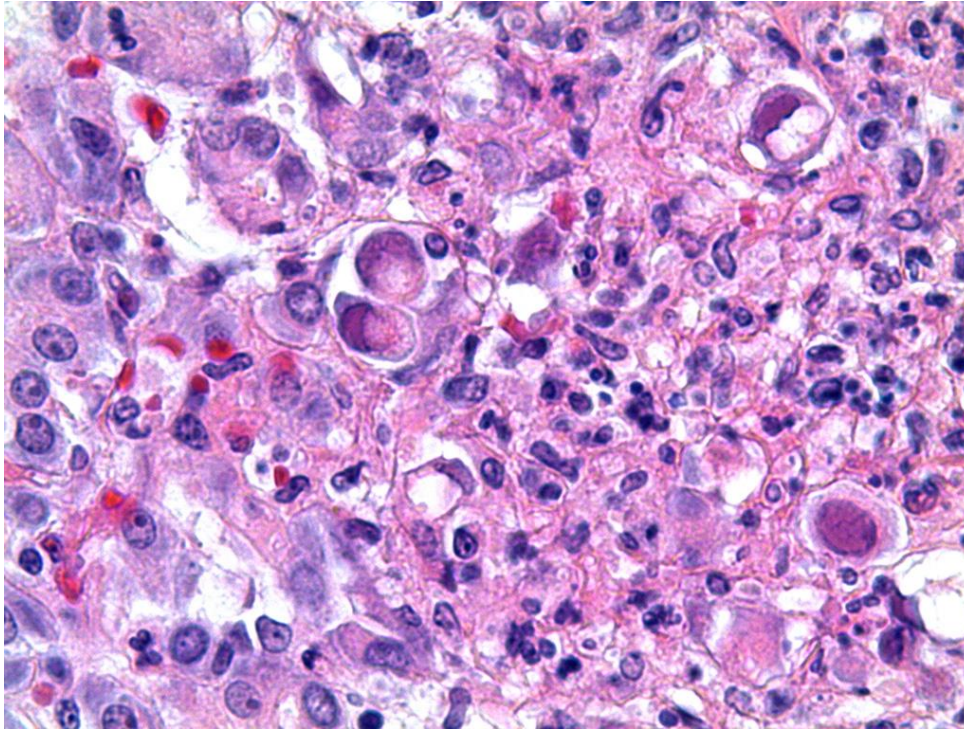


Pancreas, rhesus macaque. There is extensive lytic necrosis of the acinar pancreas with loss of architecture and infiltration of numerous viable and degenerate neutrophils, admixed with abundant cellular debris (HE, 260X)

More specific to simian research, pancreatitis is rarely reported but half the cases are associated with adenovirus.⁴ We have found most adenoviral infections to be associated with SIV infection (91%) with 45% of these presenting with acute pancreatitis (unpublished). Adenoviral replication with movement of virus to the cytoplasm accounts for the extensive liver necrosis observed in chick embryos¹ and

deficient mice exhibited enhanced disease due to blockade of endosome maturation suggesting that Rab7 may be a therapeutic target.

JPC Diagnosis: Pancreas: Pancreatitis, necrotizing, diffuse, severe with mild necrotizing steatitis and ductal and acinar intranuclear basophilic viral inclusion bodies, Indian rhesus macaque (*Macaca mulatta*), primate.



Pancreas, rhesus macaque. Degenerating acinar cells are karyomegalic with single large glassy basophilic intranuclear adenoviral inclusion. (HE, 400X)

given the large amounts of virus detected by IFA in the submitted case, it is not hard to speculate that cell lysis could easily overwhelm the protective inhibitors in tissue fluids.

Recent work with an acute pancreatitis mouse model induced by starvation has shown a critical role for small GTPase Rab7 in intracellular vesicle transport of lysosomes involved in autophagy and endocytosis.⁸ Pancreas-specific-Rab7-

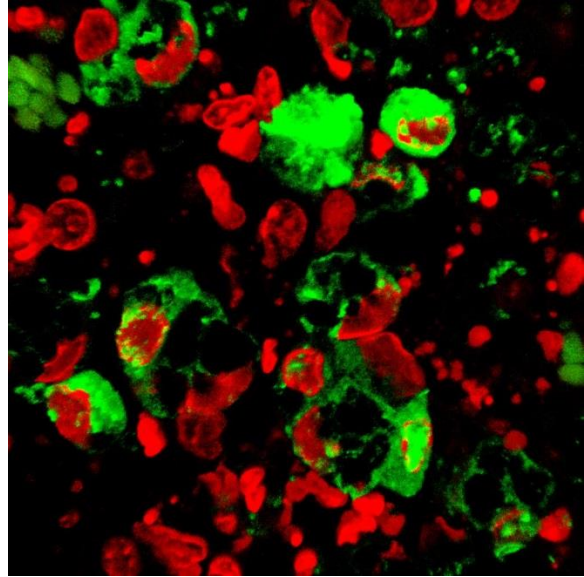
Conference Comment: Within the family Adenoviridae, of which there are numerous ubiquitous viruses that affect a wide number of species, the genus *Mastadenovirus* contains the human and nonhuman primate isolates. The name “adenovirus” is derived from adenoid, meaning the lymphoid tissue of the nasopharynx, because the first

isolates were obtained from those locations in military recruits suffering from upper respiratory tract infections. There are over 50 serotypes of adenovirus that has been isolated from nonhuman primate species (macaques, African green monkeys, baboons, chimpanzees, gorillas, orangutans, squirrel monkeys, owl monkeys, and cotton-topped tamarins), and they tend to cause respiratory or enteric disease in immune suppressed animals. In fact, adenoviruses have been isolated from healthy animals,

suggesting that persistent infections are common.⁹

When on immunosuppressive drugs or infected concomitantly with immunosuppressive viruses (SIV and betaviruses) respiratory tract infections with adenovirus results in necrosis of epithelial cells of the trachea, bronchi, bronchioles, and alveoli. The gastrointestinal tract is the second most common organ system infected, characterized microscopically by mucosal erosion or ulceration with necrotic enterocytes containing prominent adenoviral inclusions. There have been numerous reports of adenoviral-induced pancreatitis which seem to occur most frequently in young macaques, as in this case, that have been severely immunocompromised by SRV-1, SRV-2, and SIV.⁴ Even less common are necrotizing lesions of the liver, kidney, and urinary bladder which microscopically appear as necrotizing hepatitis, tubulointerstitial nephritis, and hemorrhagic cystitis with the aforementioned intranuclear inclusions. Although these inclusions are prominent in infected animals, they may resemble CMV and SV40 which also produce basophilic intranuclear inclusions, albeit resulting in significant nucleomegaly and cytomegaly.⁹

Conference participants commented on additional changes within these tissues. Within the remaining areas of intact pancreas (which were rare), there was evidence of acinar atrophy with cell shrinkage, loss of zymogen granules, and an overall increase in the acinar luminal size. , Vasculitis and thrombosis was identified in and away from areas of necrosis. In areas of necrosis, it was easier to attribute vascular necrosis to the ongoing devastation in the surrounding tissues. In areas away from the necrosis, participants searched for adenoviral inclusions within endothelium,



Pancreas, rhesus macaque, IFA. Adenovirus (green) demonstrated in nuclei and cytoplasm of many epithelial cells. Stains: anti-adenovirus IgG2, Chemicon cat#MAB8052, secondary goat-anti-mouse IgG1 with Alexa 488 Life Technologies cat# A21121, Topro3 counter stain (red) Thermo Fisher.

but none were identified. Finally, participants identified changes within the adjacent pancreatic lymph node which included edema and increased numbers of neutrophils within cortical and medullary sinuses, but rather than suggest a separate necrotizing process in the lymph node, participants decided that the lymph node was simply draining the adjacent area of inflammation.

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CASE IV: 401343 (JPC 4103279).

Signalment: 4-year-old, female, Scottish blackface sheep, *Ovis aries*, ovine.

History: The animal was referred for ill thrift and respiratory distress.



Presentation, sheep. Tipping the JRSV-infected sheep with placement of head downwards (“wheelbarrow test”) releases large amounts of clear, white fluid from the respiratory tract. (Photo courtesy of: Division of Pathology, Public Health and Disease Investigation, Veterinary Diagnostic Services, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow (Garscube Campus), 464 Bearsden Road, Glasgow G61 1QH, Scotland, <http://www.gla.ac.uk/schools/vet/>)

Gross Pathology: This animal is in poor body condition (BCS: 1.5/5). Small amounts of white froth are noted at the nares and within the lumen of the trachea. In a multifocal to coalescing distribution and affecting predominantly the right middle and caudal pulmonary lobes, the pulmonary parenchyma is effaced by multiple, reasonably well-demarcated, pale pink to grey, firm masses. On cut surface, small amounts of clear fluid run from the surface of the masses, which have a grey, granular appearance.

Laboratory results: None provided.

Microscopic Description: Lung: Affecting approximately 50% of the pulmonary parenchyma are multiple, well-demarcated, yet infiltrative, moderately to highly cellular, multifocal to coalescing neoplastic masses composed of epithelial cells arranged in a predominantly lepidic, rarely acinar or even papillary pattern, which are supported by small amounts of poorly to moderately cellular collagenous stroma. Lining the alveoli is an, in most cases, single layer of cuboidal to columnar neoplastic cells with distinct cell borders, moderate to large amounts of eosinophilic, often also vacuolated cytoplasm, a round to oval basal nucleus with rosey, clumped and vesiculated chromatin and a small, basophilic nucleolus. There is minimal to mild anisocytosis and anisokaryosis, and four mitotic figures are present in 10 HPF, some of which are bizarre. Multifocally present within the lumina of the neoplastic-lined alveolar spaces or acini are small to moderate amounts of pale eosinophilic material and small numbers of neutrophils, macrophages, occasional small to moderately sized accumulations of extravasated erythrocytes (hemorrhage) and sloughed epithelial cells. The supporting stroma multifocally exhibits mildly dispersed collagenous fibers (edema), small numbers of extravasated red blood cells (hemorrhage) and in a multifocal to coalescing distribution contains small to moderate numbers of lymphocytes, plasma cells and smaller numbers of neutrophils. The alveolar spaces in the periphery of the neoplastic nodules also contain moderate numbers of alveolar macrophages, smaller numbers of neutrophils, occasional lymphocytes and multifocally, moderate to large numbers of extravasated erythrocytes. Multifocally, surrounding the bronchioles are small to moderate numbers of lymphocytes and plasma cells in a multifocal to coalescing distribution.



Lung sheep. The cut section of the affected lung in this animal exhibits multifocal to coalescing, small, grey, granular masses. (Photo courtesy of: Division of Pathology, Public Health and Disease Investigation, Veterinary Diagnostic Services, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow (Garscube Campus), 464 Bearsden Road, Glasgow G61 1QH, Scotland, <http://www.gla.ac.uk/schools/vet/>)

Contributor's Morphologic Diagnosis:

Lung: Pulmonary adenocarcinoma, multifocal to coalescing.

Contributor's Comment: Ovine pulmonary adenocarcinoma (OPA; also known as Jaagsiekte or ovine pulmonary adenomatosis) is an infectious and contagious neoplastic disease caused by a beta retrovirus, Jaagsiekte sheep retrovirus (JSRV), an enveloped RNA virus that primarily targets sheep but also, rarely goats. This disease is present worldwide, and is particularly common in South America, South Africa and Scotland with certain breeds of sheep exhibiting a predisposition. It is absent in Australia and New Zealand and has been eradicated in Iceland.^{5,9}

Affected animals typically present with progressive dyspnea, tachypnea, nasal discharge, coughing and weight loss.⁵ A characteristic feature of this disease is the production of excessive amounts of frothy to milky fluid (surfactant proteins) from the nostrils especially when the head is lowered (wheelbarrow test), although it has been

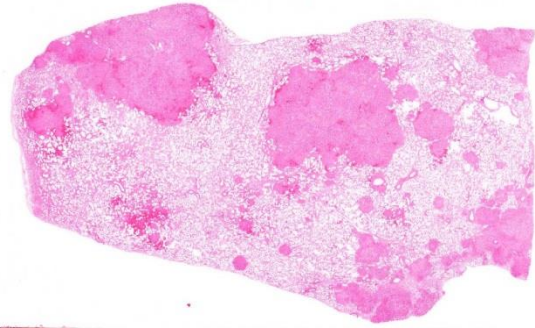
reported the amounts of fluid produced can be variable between individuals.⁷

Multifocal or locally extensive neoplasms are found in the pulmonary parenchyma with metastases to the regional lymph nodes occurring in approximately 0.3-25% of cases. More distant metastases are rare but when present, they can spread to different organs with in order of frequency: liver, kidneys, skeletal muscle, digestive tract, spleen, skin and adrenal glands.¹¹

JRSV induces oncogenic transformation of type II pneumocytes, Clara cells and progenitor cells of the pulmonary airway epithelia^{8,9}, however the exact pathogenic mechanisms of viral neoplastic transformation are poorly understood. It has been reported that attachment of the virus to the host cell is mediated through the binding of the SU subunit of the viral Env protein (envelope protein) to a specific cell surface receptor, Hyal2 on the host cell^{9,12}, which in turn mediates the entry of the virus into the cell via endocytosis.³ As with all RNA viruses, reverse transcription, where the single-stranded RNA genome is converted into a double-stranded, DNA genome, then takes place within the cytoplasm, which is essential for integration of the virus into the host genome.⁹ The Env protein has been shown to be able to induce similar tumors in immunodeficient mice and has also been shown to induce oncogenesis in rat fibroblasts, however as stated previously, the mechanisms are not clearly known.¹²

Spread of JRSV between sheep occurs through respiratory secretions from affected sheep. Additionally, lambs can be infected through their dams from the ingestion of infected colostrum.⁴

Two forms of OPA have been described, the classical and the atypical form. In the



Lung, sheep. The submitted section of lung contains numerous well-demarcated neoplastic nodules ranging up to 5mm in diameter. (HE, 5X)

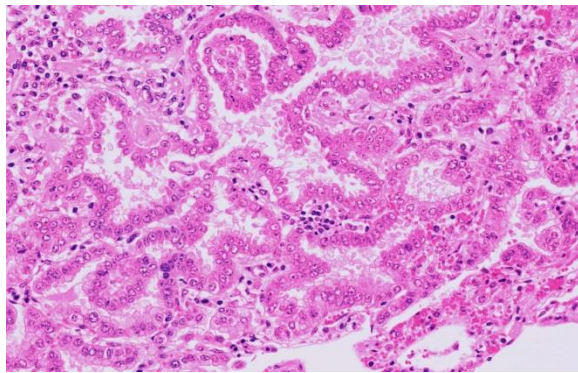
classical form, sheep present with the clinical signs as described above and typically contain multifocal to coalescing neoplastic lesions in the cranioventral pulmonary lung lobes. Grossly, the lungs are heavy, wet and fail to collapse. On cut surface, the tumors often have a grey, granular appearance and large amounts of fluid run from the surface. In contrast, the atypical form often follows a subclinical course and grossly, neoplasms are seen in the diaphragmatic lung lobes. Tumors in the atypical form are dry, white and have a multifocal distribution.^{9,11}

Microscopically, the histopathological features of both the classic and the atypical form are similar with several patterns including the lepidic pattern where the alveoli are lined by either cuboidal or columnar neoplastic cells. Other patterns include the papillary and acinar patterns.^{5,9} The atypical form differs from the classical one by having more well-demarcated neoplastic lesions which are associated with larger numbers of mononuclear cells, in particular CD4 and CD8 T-cell subsets, as well as increased amounts of fibrous tissue. It has been suggested that infiltration of mononuclear cells into the tumors of atypical cases may be due to an immunological response to tumorigenesis, whereas in the classical form, there is suppression of such infiltration.^{2,13}

Neoplastic processes can be complicated by the presence of concurrent infections including Maedi-Visna, a viral infection caused by a non-oncogenic retrovirus and resulting in interstitial pneumonia with the formation of prominent lymphoid nodules as well as interstitial fibrosis and hypertrophy of smooth muscle. In addition, bacterial infections are prevalent in a large number of sheep affected by OPA, such as those caused by *Mycoplasma* spp, and may contribute to death of the animal.^{6,9}

Although rare, non-viral pulmonary carcinomas are difficult to distinguish from ovine pulmonary adenocarcinoma based on the gross and histological lesions alone and immunohistochemistry is required to detect the JSRV envelope glycoprotein for definitive confirmation.⁵

Clinically, a further viral-induced epithelial tumor, however present in the nasal cavity, can produce similar clinical signs of increased fluid production from the nostrils. The enzootic nasal tumor is also caused by a retrovirus, the enzootic nasal tumor virus type 1 (ENTV-1) in sheep and the enzootic



Lung, sheep. Neoplastic columnar epithelium is arranged in a lepidic pattern along alveolar septa and occasionally form papillary projections into alveolar lumina. There is infiltration of low to moderate numbers of lymphocytes, macrophages, and few neutrophils within the edematous stroma. (HE, 256X)

nasal tumor virus type 2 (ENTV-2) in goats, which infects the secretory epithelial cells of the nasal glands and here leads to the formation of nasal epithelial masses with abundant secretion of mucus.^{9,14}

JPC Diagnosis: Lung: Pulmonary adenocarcinoma, Scottish blackface sheep (*Ovis aries*), ovine.

Conference Comment: Jaagsiekte sheep retrovirus (JSRV) belongs to the family *Retroviridae*, subfamily *Orthoretrovirinae*, and genus *Betaretrovirus*. JSRV (as described above) is the causative agent of contagious lung tumors in sheep called ovine pulmonary adenocarcinoma. The term “jaagsiekte” is taken from the Afrikaans words for “chase” (jag) and “sickness” (siekte) describing the common clinical scenario of sheep that exhibit respiratory distress from being chased. The infectious “exogenous” form of JSRV has an endogenous counterpart (enJSRVs) which is present in the genome of healthy sheep and goats.¹⁵ Sheep have approximately 27 copies of enJSRVs that are able to block the JSRV replication cycle and have an essential role in fetal development and formation of the placenta.¹

JSRV is transmitted via respiratory droplets and infect respiratory epithelial cells (type II pneumocytes or Clubb cells) as well as lymphocytes and myeloid cells, but replicates most in alveolar type II pneumocytes and Club cells.¹⁰ This virus has the typical “gag”, “pol”, and “env” genome arrangement bordered on each end by a long terminal repeat (LTR). The “gag” portion encodes the internal structural matrix, capsid, and nucleocapsid proteins. “Pol” encodes the RT and integrase enzymes. Most importantly, “env” encodes surface and transmembrane envelope glycoproteins which aid in viral cellular

entry and directly stimulate neoplastic transformation of type II pneumocytes and/or Club cells.⁹

An interesting feature of JRSV infection is lack of host immune response. There are two possible explanations for this phenomenon. (1) The sheep may be immunologically tolerant to JRSV due to the already present enJRSV that would be expressed in the fetal thymus during T-cell development. Thus, any anti-JRSV reactive T-cells would be removed via self-tolerance mechanisms. (2) Another possibility is that tumor cells downregulate their expression of MHC-I. This hypothesis is supported by research that has identified an absence of virus-specific cytotoxic T-cells.⁹

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