Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #18 **1 February 2023**

CASE I:

Signalment:
Goitered gazelle (*Gazella subguttorosa*), adult, gender unknown.

History:
Lung tissue was collected from a carcass found in Darvi soum, Khovd province, Mongolia in January 2017. Formalin-fixed tissue was embedded in paraffin at the State Central Veterinary Laboratory, Mongolia.

Gross Pathology:
The carcass was emaciated.

Laboratory Results:
The lateral flow device penside test for eye swab was positive for Peste-des-petits-ruminants virus antigen.

Microscopic Description:
Mild epithelial desquamation and peribronchial edema are observed in the bronchi. Epithelial necrosis and desquamation are severer in bronchioles. Infiltration of neutrophils, epithelioid macrophages and syncytial cells occurs multifocally in alveolar lumina, which is associated with the proliferation of type II pneumocytes. Bronchial and bronchiolar epithelial cells sometimes contain intracytoplasmic and intranuclear inclusion bodies. Intracytoplasmic and intranuclear inclusion bodies are also frequently observed in epithelioid macrophages and syncytial cells.

By immunohistochemistry, bronchial and bronchiolar epithelial cells, epithelioid macrophages, syncytial cells, and type II pneumocytes reacted weakly with anti-canine distemper virus monoclonal antibody (clone DV2-12).

Contributor's Morphologic Diagnoses:
Lung: Acute necrotizing bronchopneumonia with intracytoplasmic and intranuclear inclusion bodies and syncytial cell formation.

Contributor's Comment:
Peste des petits ruminants (PPR) is a viral disease of goats and sheep characterized principally by stomatitis, diarrhea, oculonasal discharge, and pneumonia. The causative agent is closely related to rinderpest virus and these two are classified along with the measles virus and CDV in the genus Morbillivirus in the Family *Paramyxoviridae*. PPR was reported to the OIE, for the first time in Mongolia, in 2016 and was confirmed to affect sheep, goat and yak in the western part of Mongolia.[2]

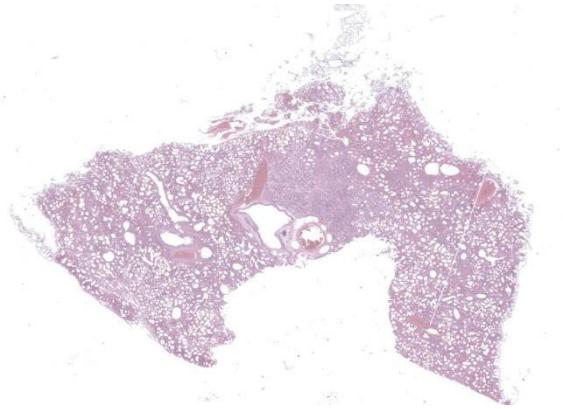

Figure 1-1. Lung, gazelle. A single section of mildly autolytic lung is submitted from examination (HE, 6X)

Death in these domestic animals declined rapidly after extensive vaccination; however, the virus continued to spread to wild animals such as saiga, Siberian ibex and goitered gazelle.[2]

Histopathological findings observed in this case are similar to those in goats and sheep experimentally infected with PPR virus.[1,3] Syncytial cells observed within alveolar spaces were reported to be macrophage origin.[3]

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JPC Diagnosis:
Lung: Pneumonia, bronchointerstitial, necrotizing, diffuse, marked, with edema, numerous viral syncytia, and intranuclear and intracytoplasmic eosinophilic viral inclusions.

JPC Comment:
This case of peste des petits ruminants virus infection in a goitered gazelle bears striking similarities to the case of canine distemper virus infection and pneumonia in Conference 17, Case 2, of this year, as they are both morbilliviruses and produce similar diseases. Peste des petits ruminants (PPR), which translates to mean plague of small ruminants, is known by several other names, including kata, stomatitis-pleuropneumonia complex, goat plague, ovine rinderpest.[4,8] Goats and sheep are the primary hosts, with goats typically experiencing more severe disease, and while other animals such as cattle, buffalo, and pigs can be infected, they are considered dead end hosts that do not shed the virus and generally do not develop clinical signs.[5]

PPR was first described in 1942 by Gargadennec and Lalanne in the Ivory Coast, West Africa, though the disease may have been present beforehand and misidentified as rinderpest virus.[5] It is now endemic in Africa,

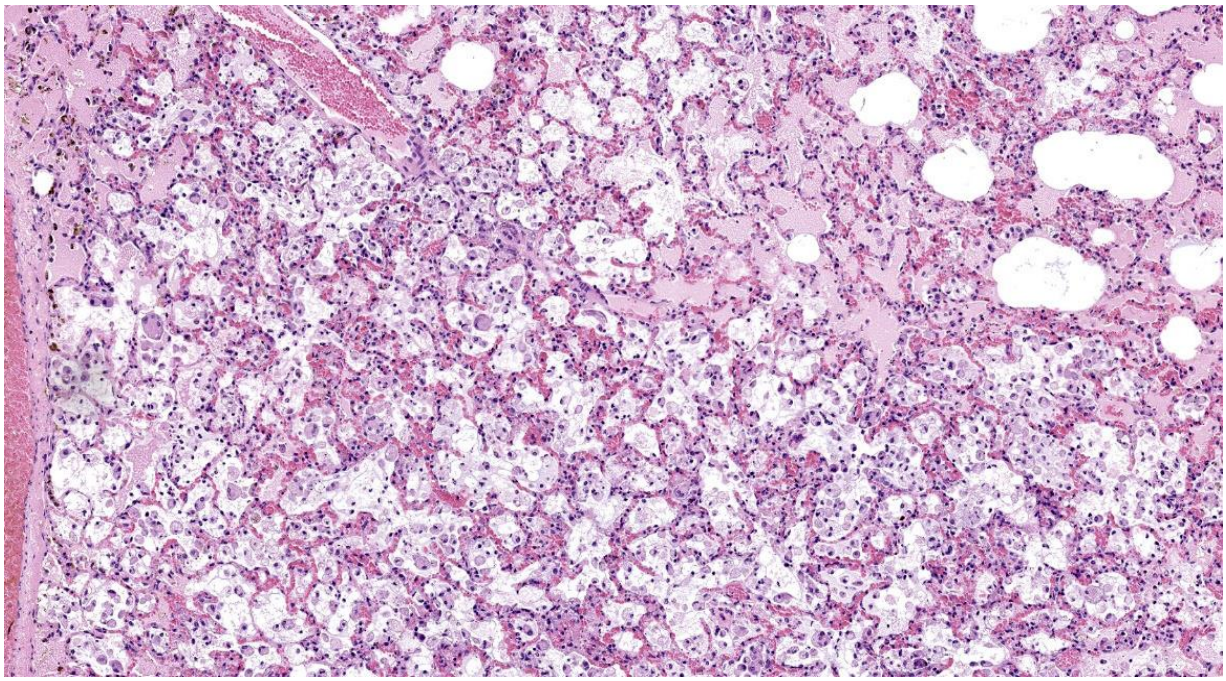

Figure 1-2. Lung, gazelle. Regionally, alveolar septa are markedly expanded, and alveoli are filled with edema fluid and numerous foamy alveolar macrophages. (HE, 109X)

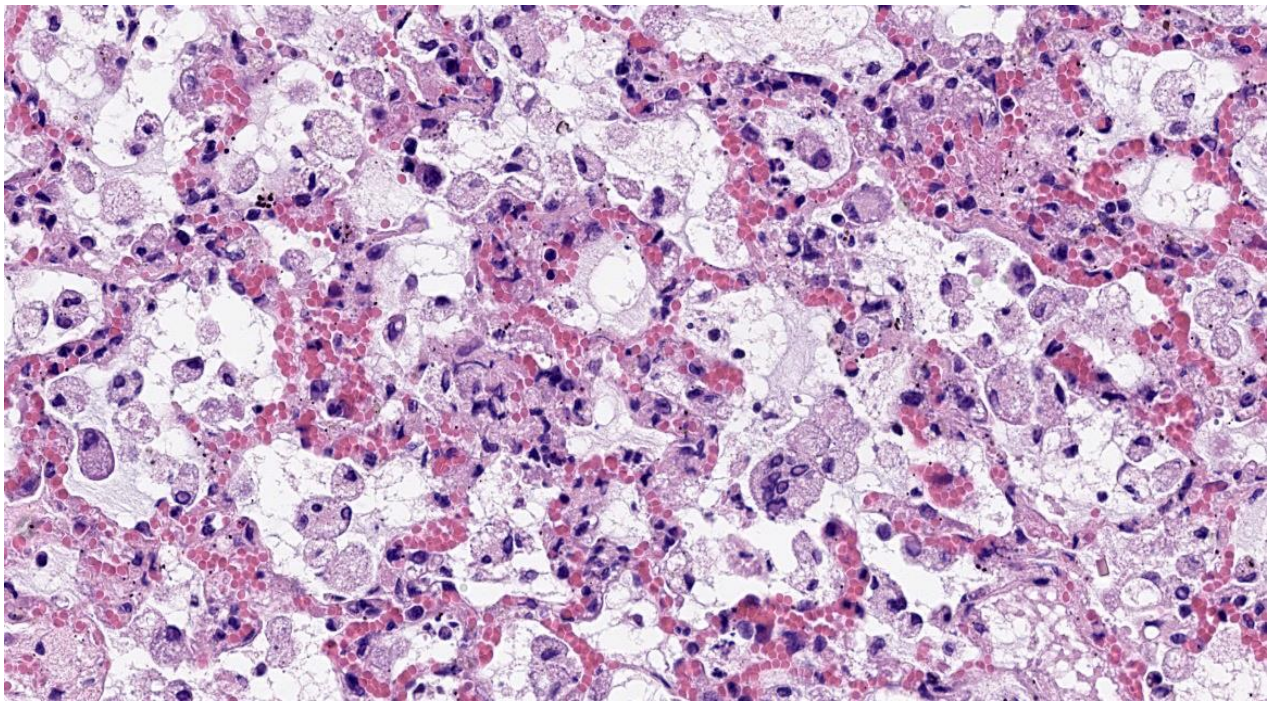

Figure 1-3. Lung, gazelle. Higher magnification of affected areas of lung. Alveolar spaces also contain scattered multinucleated syncytia (arrow). (HE, 381X)

the Middle East, China, India, and south Asia. There are four lineages of the virus, and while historically lineages I, II, and III have been present in Africa and IV present in Asia, lineage IV has now become the dominant strain in Africa.[5] Increased incidence has been associated with the spread of lineage IV, possibly indicating that is more virulent.[5]

PPRV is a non-segmented, single-stranded, negative-sense morbillivirus, and like other morbilliviruses, is highly lymphotropic and epithliotropic.[4,5] The virus uses the hemagglutinin protein H to bind to the typical morbillivirus receptors: signaling lymphocyte activation molecule (SLAM)/CD150 on lymphocytes, macrophages, and dendritic cells; and Nectin-4 on epithelial cells.[4,5] The F protein facilitates fusion of the viral envelop and host cell membrane, and the virus subsequently replicates in the cytoplasm.[5] The M protein facilitates viral egress from host cells via budding.[5]

Infection occurs through direct contact with aerosolized virus or infected bodily secretions. It is presumed that the virus first infects leukocytes in the respiratory mucosa and then spread to local lymphoid tissue where initial replication occurs. After an initial immune cell proliferation, viremia results in systemic spread of the virus, and

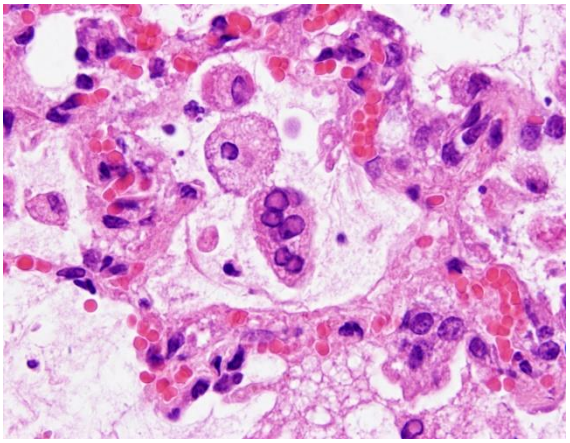

Figure 1-4. Lung, gazelle. Alveoli contain multinucleated viral syncytia with intranuclear and intracytoplasmic inclusions. Intracytoplasmic inclusions are also present within the cytoplasm of uninucleate alveolar macrophages. (HE, 400X) (Photo courtesy of: Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan, https://www.vetmed.hokudai.ac.jp/en/)

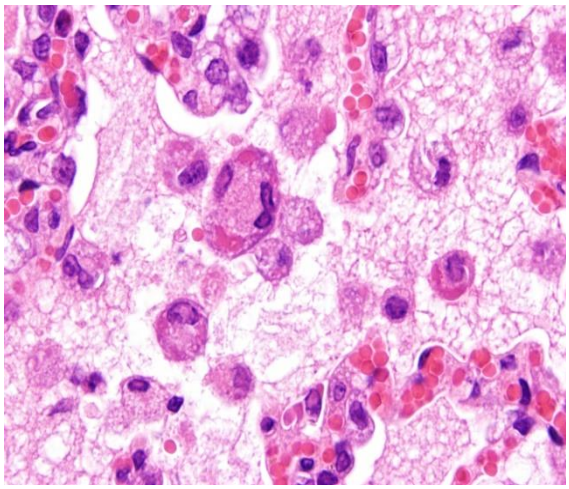

Figure 1-5. Lung, gazelle. Another field more clearly demonstrates intracytoplasmic viral inclusions within alveolar macrophages. (HE, 400X) (Photo courtesy of: Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan, https://www.vetmed.hokudai.ac.jp/en/)

subsequent leukopenia with reduction of CD4+ T cells occurs approximately 4 days post infection. PPRV causes clinically significant immunosuppression by inducing leukopenia and inhibition of type I and II interferon activity due to viral nonstructural proteins.[5]

PPRV has high morbidity and mortality rates, though certain breeds are more resistant and virulence varies by viral strain.[5] The disease has a more rapid onset and clinical progression than rinderpest.[8] Clinical signs begin around 3 days post-infection and initially consist of pyrexia, malaise, and serous to mucopurulent oculonasal discharge, and results in dyspnea.[4] In animals which recover, clinical signs resolve by 14 days post-infection, and these animals generally have life-long immunity.[4]and pseudomembranous ulcerations in the mouth and nose, followed by acute severe gastroenteritis and hemorrhagic colitis.[5,8] Pneumonia occurs late in the disease process

Profound immunosuppression can lead to secondary bacterial infection by microbes such as *Mycoplasma* or *Pasteurella.*[4,8] This week's moderator, Dr. Kali Holder from the Smithsonian's National Zoo, described how morbilliviruses cause immunosuppression by causing leukopenia, by targeting mechanisms of innate immunity, and by inducing immune amnesia by specifically targeting T and B memory cells and dendritic cells.

Histologic features of PPRV infection are typical for morbilliviruses. Syncytia form in leukocytes of the lymph nodes, white pulp, GALT, oral mucosa, pulmonary alveoli, and liver.[4,8] Eosinophilic cytoplasmic and nuclear inclusions occur in the renal pelvis, abomasum, respiratory epithelium, and type II pneumocytes.[8] Histologic lesions in the lung include inflammation and necrosis of airway epithelium, bronchointerstitial pneumonia, and type II pneumocyte hyperplasia, as seen in this case.[1,8]

This week's moderator also described how PPRV has caused massive mortality events in hoofstock in Mongolia, including the Siberian ibex, the goitered gazelle, and in the critically endangered Saiga antelope. Researchers using distance sampling techniques

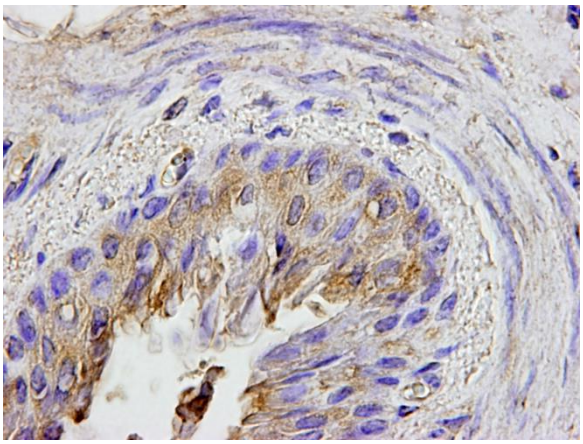

Figure 1-6. Lung, gazelle. Hyperplastic airway epithelium is strongly immunopositive for PRRV antigen. (anti-PPRV, 400X) (Photo courtesy of: Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan, https://www.vetmed.hokudai.ac.jp/en/)

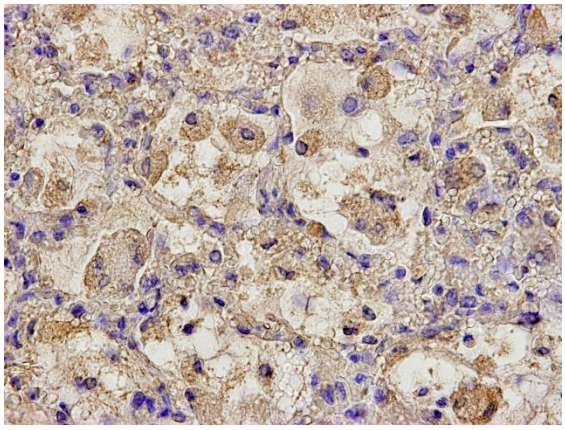

Figure 1-7. Lung, gazelle. Alveolar macrophages are strongly immunopositive for PRRV antigen. (anti-PPRV 400X) (Photo courtesy of: Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan, https://www.vetmed.hokudai.ac.jp/en/)

estimate that PPRV outbreak caused a population decline of up 80% in the Saiga antelope.[6]

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CASE II:

Signalment:
One-month-old, male intact Grant's gazelle (*Nanger granti granti*)

History:
Acute onset of lethargy, anorexia, and pyrexia (106 F°). After initial supportive care, euthanasia was elected.

Gross Pathology:
An approximately 10 x 10 cm area of the right lobe of the liver surrounding the gallbladder had a roughened, red to tan granular capsular surface. On cut section, the subtending parenchyma was granular and sharply demarcated from the left lobe. The lungs were diffusely dark red, wet, and heavy.

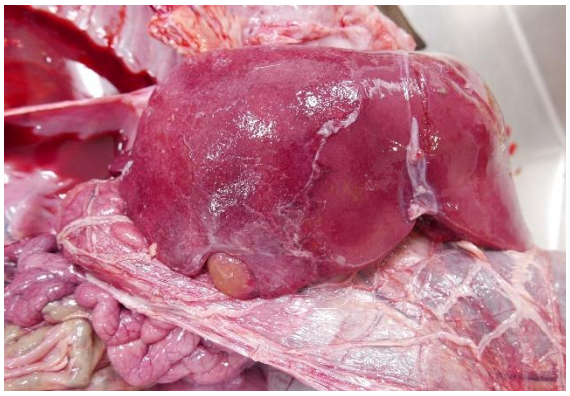

Figure 2-1. Liver, Grant's gazelle. The liver has a regionally extensive area near the gallbladder with a granular capsular surface. (Photo courtesy of: Disease Investigations, San Diego Zoo Wildlife Alliance, http://institute.sandiegozoo.org/disease-investigations)

Laboratory Results:

PCR: A sample of liver was submitted for Chlamydia PCR and was positive. Sequencing identified *Chlamydia pecorum*.

Microscopic Description:

Liver: Within a well-demarcated region of the liver are multifocal distinct and occasionally coalescing foci of neutrophilic inflammation, karyorrhectic debris, and rare multinucleated giant cells that obscure the normal architecture. Neutrophilic aggregates are surrounded by a rim of hepatocytes with a loss of nuclear detail and faint cytoplasmic borders (coagulative necrosis) and acute hemorrhage with fibrin. Kupffer cells adjacent to areas of inflammation and necrosis occasionally have granular amphophilic material in the cytoplasm (coccobacilli). Diffusely, intact hepatocytes have variably sized, well-defined intracytoplasmic vacuoles, and sinusoids are mildly congested. Multiple portal areas have a mild increase in biliary epithelial cells (ductular reaction).

Immunohistochemistry (Chlamydia): Clusters of punctuate, intra-cytoplasmic immunoreactivity is common in areas of necrosis and less common within areas of suppurative inflammation. No immunoreactivity is detected with Coxiella IHC.

Contributor's Morphologic Diagnoses:

1. Liver: Severe, acute, regionally extensive, necrosuppurative hepatitis with intralesional coccobacilli, *Chlamydia pecorum*

Contributor's Comment:

Chlamydia pecorum is one of eleven species within the Chlamydia genus, the only genus in the family *Chlamydiaceae*.[12] It is endemic in many populations of cattle and sheep, as detection in the intestines of clinically normal animals is common.[1,7] The bacterium has been reported to cause disease in a wide range of species, but koalas, various species of domestic and exotic ruminants, and swine appear to be the most commonly affected. In ruminants, particularly lambs and calves, polyserositis including arthritis, encephalomyelitis, keratoconjunctivitis, mastitis, orchitis, placentitis, endometritis, and pneumonia have been reported.[1,6,8,14] Additionally, one study described up to a 48% reduction in gained weight in infected calves suggesting the economic impact extends beyond individuals with clinical disease.[10] While less abortogenic than *C. abortus*, necrotizing placentitis and neutrophilic hepatitis have also been described in goats and sheep that aborted due to *C. pecorum* infection.[5,15] This case is unusual in that the liver was the organ affected in this one-month-old. Most likely, the liver was infected via portal spread from the small intestine. The source of infection in this case is also unknown, as this is the first report of disease caused by *C. pecorum* at the institution. Spread from nearby domestic animals or direct exposure from subclinically infected conspecifics are both possible.

In koalas, *C. pecorum* can result in keratoconjunctivitis as well as disease in the urogenital and respiratory tracts resulting in blindness, infertility, or a condition known as 'wet bottom' or 'dirty tail' due to urine staining of the fur secondary to cystitis and urinary

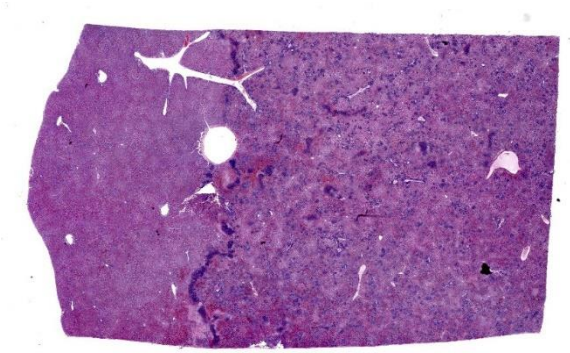

Figure 2-2. Liver, gazelle. At subgross magnification, coalescing areas of necrosis in both sections are visible. (HE, 8X)

incontinence.[1,8] Conjunctivitis due to *C. pecorum* infection in koalas in Southern Australia has been associated with koala gammaretrovirus infection (KoRV-B).[4]

Of the Chlamydia spp. that cause disease in animals, *C. psittaci* is the most well-documented zoonotic species and can be cause mild conjunctivitis (ornithosis) or more severe disease due to myocarditis, encephalitis, or pneumonia.[1,12] The zoonotic potential of *C. pecorum* is less clearly defined.[3]

A recent review describes the challenges of diagnosing chlamydial diseases in animals.[1] While neutrophilic inflammation, necrosis, and basophilic 'chlamydial' inclusion bodies are considered histologically characteristic, many infections lack these features or have less specific inflammatory pattern. As a result, immunohistochemistry, histochemical staining, and molecular tests are considered the mainstays of diagnosis of chlamydial diseases in animals. Histochemical stains to highlight the intracellular bacteria via cytology or histology include modified Macchiavello, Giemsa, Castaneda, modified Ziehl-Neelsen, or modified Gimenez.[11] Molecular techniques are considered the gold standard for diagnosis due to the inability to isolate *Chlamydia* spp. on agar plates as they require a host cell for survival. However, care should be taken not to over interpret a positive PCR test as infection with *C. pecorum* is considered endemic in many ruminants and may be an incidental finding in the absence of associated disease.[7] Fluorescent antibody tests and ELISAs also have been developed but are unable to differentiate between various *Chlamydia* species.[11] Immunohistochemistry and PCR were both used in this case to confirm *C. pecorum* as the causative agent.

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San Diego, CA 92112
http://institute.sandiegozoo.org/disease-investigations

JPC Diagnosis:
Liver: Hepatitis, necrotizing, focally extensive, marked, with intrahepatocytic and intrahistiocytic bacteria.

JPC Comment:
Chlamydia species are obligate intracellular Gram-neagative bacteira with a unique lifecycle which begins as an infectious extracellular elementary body.[1,2] Inside the host cell, the bacteria matures within a vacuole into a slightly larger reticulate body, and after several rounds of replication, the bacteria converts back to an elementary body and lyses the cell.[1,2] During times of stress, the bacteria may take on an aberrant body phenotype, a non-replicative persistent state.[1]

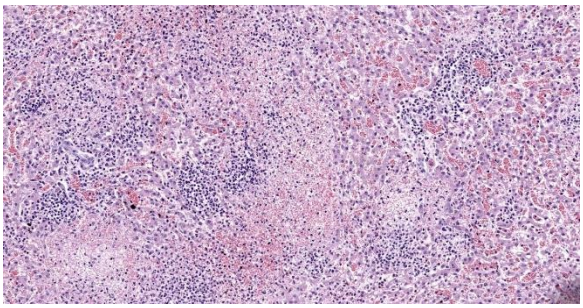

Figure 2-3. Liver, gazelle. Higher magnification of areas of necrosis, showing predominantly lytic foci. (HE, 792X)

While the contributor describes several forms of Chlamydial infection in mammals, the bacteria also causes clinical and subclinical infection in reptiles, amphibians, and fish. In reptiles, clinical disease is characterized by granulomatous inflammation in the heart, lung, liver, and/or spleen.[1,2] A recent article in *Vet Pathol* described an outbreak of *Chlamydia* in farmed American alligators.[2] Over the span of ten days, approximately 100 alligators died acutely. Histologically, these animals had severe necrotizing hepatitis and myocarditis, mild enterocolitis, and splenic lymphoid depletion; most animals also had lymphoplasmacytic interstitial pneumonia and nephritis.[2] Animals which died later during the outbreak also had erosive conjunctivitis, keratoconjunctivitis, and mild uveitis; lesions in other systems were less severe than in the animals that died earlier in the outbreak.[2] Chlamydial antigen was identified using immunohistochemistry and was particularly high in the liver, heart, spleen, and intestine.[2] Based on genetic sequencing and phylogenetic analysis, the authors suspect that the *Chlamydia* species is closely related to *Chlamydia poikilothermis* identified in snakes.[2]

An important differential to consider for this case of well-demarcated, randomly distributed foci of hepatic necrosis in a ruminant is ruminal acidosis with secondary necrobacillosis (rumenitis-liver abscess complex). [13]

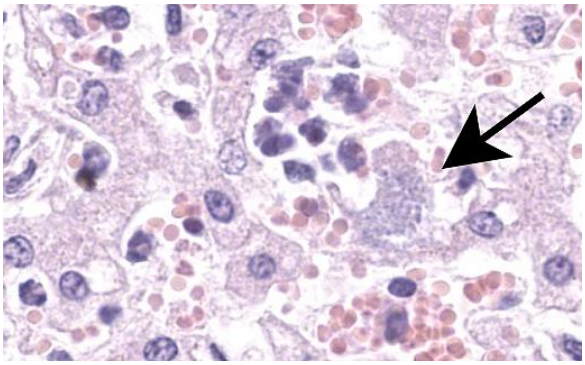

Figure 2-4. Liver, gazelle. Rare hepatocytes and Kupffer cells contain cytoplasmic bacteria (arrow). (HE, 150X)

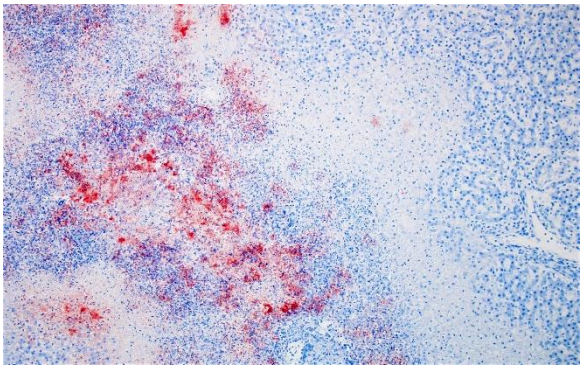

Figure 2-5. Liver, gazelle. There is strong immunoreactivity to antibodies against Chlamydia sp. within areas of necrosis. (anti-Chlamydia sp., 100X) (Photo courtesy of: Disease Investigations, San Diego Zoo Wildlife Alliance, http://institute.sandiegozoo.org/disease-investigations)

Ruminal acidosis is caused initially by carbohydrate overload and leads to alterations of microflora, overgrowth of organisms such as *Streptococcus bovis*, and ruminal atony.[13] High levels of acid within the rumen and intestine cause increased osmotic pressure, fluid influx, and systemic hypovolemia. Ultimately, *Fusobacterium necrophorum*, an opportunistic pathogen which is part of the normal GI flora, invades the ruminal mucosa, causing mucosal necrosis and ulceration, and travels through the portal system causing multifocal random hepatic necrosis.[13] While the pattern of necrosis in this case is consistent with necrobacillosis, this case lacks colonies of filamentous bacteria along the margins of necrosis seen with *F. necrophorum.*

The moderator and conference participants agreed that, while intracellular bacteria were visible on the H&E slide, reaching a definitive diagnosis of *Chlamydia pecorum* is not possible without further testing (such as with PCR and IHC, as completed in this case.) Other differentials for intracellular bacteria discussed by the participants included *Brucella* and *Clostrium piliforme.*

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CASE III:

Signalment:
A 5-year-old, female, bald eagle (*Haliaeetus leucocephalus*).

History:
The bald eagle was found on the side of the road and looked like it couldn't walk properly. On arrival at the LSU Wildlife Hospital, the bald eagle was emaciated, lethargic, and anemic (PCV: 23%). The left metacarpal joints were swollen and had purulent exudates. She had severe louse infestation, and fecal flotation revealed whipworm eggs and strongyle eggs. The eagle was treated with fluids, Frontline, vitamin B complex, iron dextran, meloxicam, ceftiofur, and piscivore

diet. Over ten days the eagle got more alert and ate fish and rabbit on her own, but then declined and found dead in her kennel, 13 days after admission.

Gross Pathology:
The bald eagle was found on the side of the road and looked like it couldn't walk properly. On arrival at the LSU Wildlife Hospital, the bald eagle was emaciated, lethargic, and anemic (PCV: 23%). The left metacarpal joints were swollen and had purulent exudates. She had severe louse infestation, and fecal flotation revealed whipworm eggs and strongyle eggs. The eagle was treated with fluids, Frontline, vitamin B complex, iron dextran, meloxicam, ceftiofur, and piscivore diet. Over ten days the eagle got more alert and ate fish and rabbit on her own, but then declined and found dead in her kennel, 13 days after admission.

Laboratory Results:
Aerobic bacterial culture (liver): *E. coli* (moderate), *Enterococcus canintestini* (moderate)
Salmonella culture (liver): Negative
Fecal flotation test: No parasites seen

Microscopic Description:

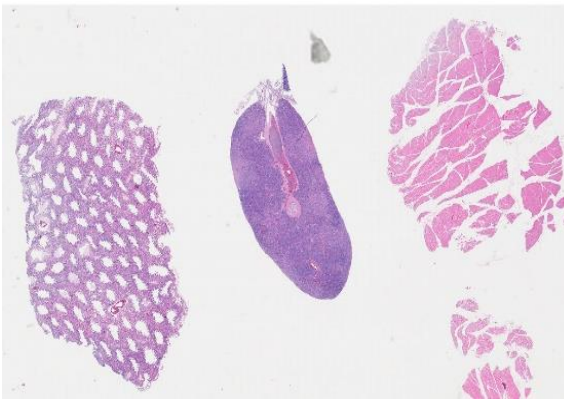

Figure 3-1. Multiple tissues, bald eagle. Lung, pancreas and skeletal muscle are submitted for examination. Loss of normal pulmonary architecture is present at subgross magnification. (HE, 6X)

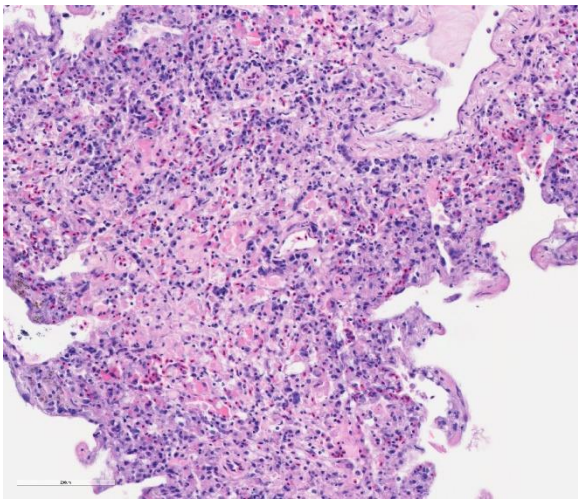

Figure 3-2. Lung, bald eagle. Normal architecture of air capillaries is effaced by a mixed inflammatory infiltrate and lumina contain abundant eosinophilic protein and fibrin. (HE, 166X)

Lung: The parabronchial wall is diffusely infiltrated by large numbers of plasma cells, lymphocytes, fewer Mott cells, and heterophils, narrowing and obliterating the air capillaries. Some air capillaries contain eosinophilic proteinaceous material mixed with fibrin. Frequently within presumed endothelial cells, there are clusters of 1-2 x 3-5 µm, elongated, basophilic, merozoites with a small dark basophilic nucleus. Occasional interatrial septa have small aggregates of macrophages containing brown-black pigment (pneumoconiosis).

Pancreas: There is multifocal to coalescing necrosis associated with multiple clusters of merozoites. Mild fibroplasia and ductular reaction are present adjacent to the necrotic areas. Low numbers of plasma cells and lymphocytes infiltrate the interstitium. The capsule is partially covered by fibrin, and the capsule abutting the necrotic area is thickened by fibroplasia and infiltration of macrophages.

Skeletal muscle: Occasional myofibers contain sarcocysts measuring up to 80 x 150 µm, containing large numbers of bradyzoites. Sporadic myofibers are degenerate and

necrotic, characterized by loss of cross-striation and fragmentation of the sarcoplasm. Occasionally, necrotic myofibers are replaced by infiltration of macrophages and lymphocytes.

Contributor's Morphologic Diagnoses:
Lung: Severe subacute diffuse lymphoplasmacytic interstitial pneumonia with intraendothelial apicomplexan merozoites
Skeletal muscle: Multiple sarcocysts; mild multifocal muscular degeneration and necrosis
Pancreas: Moderate subacute multifocal to coalescing necrotizing pancreatitis with intralesional apicomplexan merozoites

Contributor's Comment:
In this case, merozoites consistent with *Sarcocystis* sp. and associated necrotizing inflammation was noted in multiple organs including the lung, liver, pancreas, cerebellum, and to a lesser extend in the cerebrum, and sarcocysts were noted in the skeletal and cardiac muscle. The final diagnosis on this case was systemic sarcocystosis. Chronic septic arthritis of the left carpal joint was also noted.

Sarcocystis spp. are apicomplexan protozoal parasites that infect mammals, birds, and reptiles. More than 200 species are known in this genus. *Sarcocystis* spp. have an obligate 2-host life cycle. The sexual stages develop in a predator host (definite host), whereas the asexual phases develop in the prey animal (intermediate host). The intermediate host ingests sporocysts or sporulated oocysts through herbage contaminated by the definite host's feces. Sporozoites are released and invade endothelial cells where they undergo schizogony. One or two generations of schizogony take place. The merozoites derived from these generations invade skeletal and cardiac myocytes where they develop into thin-walled cysts initially containing round metrocytes, which repeatedly divide through

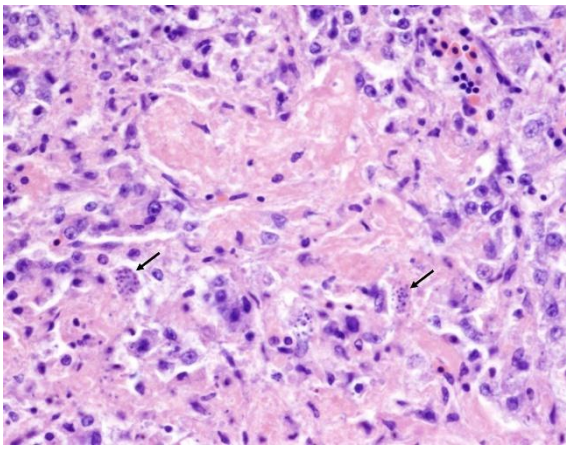

Figure 3-3. Lung, bald eagle. Endothelial cells contain numerous 2-3um apicomplexan merozoites (arrows). (HE, 400X) (Photo courtesy of: Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA. https://www.lsu.edu/vetmed/laddl/)

a process termed endodyogeny to produce numerous banana-shaped bradyzoites. The definite host consumes the skeletal muscle with the sarcocysts containing bradyzoites. The sexual cycle is developed in the intestinal epithelium of the predatory host.[5,9]

In most cases, sarcocystosis is subclinical in the definitive or intermediate host. In definitive hosts, it causes intestinal sarcocystosis which is often asymptomatic or self-limiting. For intermediate hosts, muscular sarcocystosis is a common incidental finding on postmortem examination. However, certain *Sarcocystis* species can cause serious diseases in intermediate hosts. Those include *Sarcocystic neurona* causing equine protozoal myeloencephalitis in horses and *S. calchasi* causing pigeon protozoal encephalitis in domestic pigeons.

Certain *Sarcocystis* spp. are zoonotic, and Sarcocystosis may be underdiagnosed in humans. Humans are definite hosts of *S. hominis, S. heydorni, and S. suihominis*. Consuming undercooked beef (*S. hominis, S. heydorni*) and pork (*S. suihominis*) is the route of infection, which causes intestinal

sarcocystosis. Humans also serve as intermediate hosts for other *Sarcocystis* spp. by accidental consumption. One example is *S. nesbitti* of which a predatory snake is thought to be the definite host. In these circumstances, muscular sarcocystosis can develop.[2,9]

At least six species of *Sarcocystis* infect birds.[11] Two species are known to cause serious clinical diseases in birds. *Sarcocystis calchasi*, the causative agent of pigeon protozoal encephalitis, has been first described in domestic pigeons and causes sporadic cases of encephalitis in Columbidae. Epizootics associated with *S. calchasi*-induced encephalitis have been reported in wild feral rock pigeons[6], in three different psittacine species in a zoological exhibit[7], and in wild Brandt's cormorants [1], all of which occurred in California. Experimental studies demonstrated a biphasic disease in domestic pigeons: an acute schizogonic phase and a late phase with neurologic signs. According to a study, inflammatory lesions in the cerebrum were identified as early as 20 days post infection (d.p.i.) while the onset of neurologic signs appeared at 47 d.p.i. As the numbers of *sarcocystis* organisms or the amount of DNA did not increase with the increase of cerebral inflammation, a delayed-type hypersensitivity was proposed as the pathogenesis of *S.*

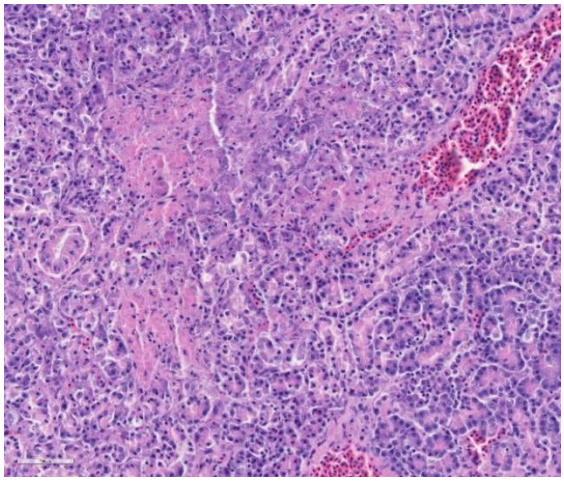

Figure 3-4. Pancreas, bald eagle. There are multifocal areas of lytic necrosis of acinar tissue. (HE, 166X)

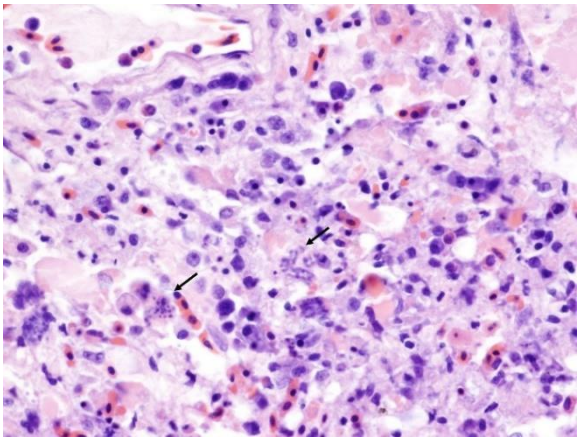

Figure 3-5. Pancreas, bald eagle. Endothelial cells contain numerous 2-3 µm apicomplexan merozoites (arrows). (HE, 400X) (Photo courtesy of: Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA. https://www.lsu.edu/vetmed/laddl/)

calchasi-induced encephalitis.[4] The definite hosts of *S. calchasi* are northern goshawks (*Accipiter gentilis*) and European sparrowhawks (*A. nisus*) in Europe and Cooper's hawks (*A. cooperii*) and red-tailed hawks (*Buteo jamaicensis*) in North America.[8]

Sarcocystis falcatula, which also can cause fatal sarcocystosis in birds, uses Virginia opossums (*Didelphis virginiana*) as a definite host. Natural intermediate hosts are cowbirds and grackles. Psittacine birds, especially old world psittacine, are known to be susceptible to *S. falcatula* infection. The primary lesion is pulmonary congestion, edema, and hemorrhage with intravascular merozoites, but myositis and encephalitis also occur. Clinical disease also has been reported in Victoria crowned pigeons characterized by sudden death caused by pulmonary sarcocystosis.[10] Birds of prey including free-ranging bald eagles, golden eagles[12], and a great horned owl[13] also have been reported with fatal pulmonary and/or neurologic sarcocystosis caused by *S. falcatula*. *S. ryleyi* affects waterfowls and usually is an incidental finding at necropsy.

According to Wünschmann et al.[12], among three bald eagles and one golden eagle that had fatal *Sarcocystis falcatula* infection, two bald eagles had blood lead concentration consistent with subclinical lead intoxication, and one had lead intoxication. It was not discussed in this article whether the lead intoxication played a role in the disease susceptibility, but we were wondering if lead intoxication make the birds more susceptible to sarcocystosis. In the present case, the infection was disseminated affecting multiple organs, compared to previously reported sarcocystosis cases in raptors. Heavy metal level was not measured in this case, but the chronic septic arthritis may have caused immunosuppression predisposing the fatal systemic sarcocystosis.

In this case, the diagnosis was made based on the morphology and the location (pulmonary endothelium) of the apicomplexan parasites, and further characterization of the organism was not performed. PCR is usually used for confirmation of sarcocystosis and identifying the species.

Contributing Institution:
Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
https://www.lsu.edu/vetmed/laddl/
https://www.lsu.edu/vetmed/pbs/index.php

JPC Diagnosis:
1. Lung: Pneumonia, interstitial, lymphoplasmacytic and histiocytic, diffuse, marked with intraendothelial apicomplexan merozoites.
2. Pancreas: Pancreatitis, necrotizing, multifocal to coalescing, moderate with intraendothelial apicomplexan merozoites.
3. Skeletal muscle: Sarcocysts, numerous.

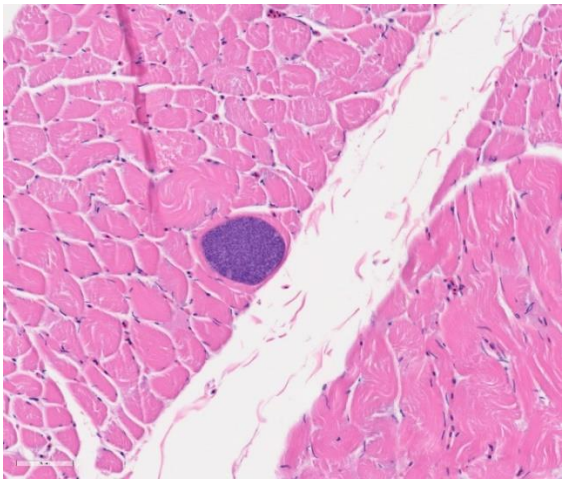

Figure 3-6. Skeletal muscle, bald eagle. Numerous myocytes contain sarcocysts which occupy part of the sarcoplasm. (HE, 166X)

JPC Comment:
As the contributor describes, *Sarcocystis calchasi* is one of the main pathogenic Sarcocystis species in birds and primarily infects Columbiformes and Psittaciformes. The first documented cases of infection in Galliformes were recently reported in a 2022 *Journal of Veterinary Diagnostic Investigation* article.[3] Two captive vulturine guineafowl, ground dwelling birds, developed acute and rapidly fatal infections after having been moved to a new enclosure with other avian species.[3] The first bird presented unable to stand and experienced progressive neurologic signs.[3] The second bird presented shortly after the first with nonspecific signs of lethargy and weight loss, and both animals died despite ponazuril therapy.[3] The animals had nonsuppurative meningoencephalitis with perivascular lymphoid cuffing and glial nodule formation, and one animal had protozoal schizonts in areas of inflammation.[3] Both animals were PCR positive for *Sarcocystis*, and sequencing indicated the species was *S. calchasi*.[3]

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CASE IV:

Signalment:
12-year-old male striped hyena (*Hyaena hyaena*)

History:
A firm, non-painful, approximately 3-cm-diameter subcutaneous mass was observed elevating skin over the dorsal cervical region. Skin was intact and freely moveable over the surface of the mass. The mass was monitored for a period of several months with no obvious changes. A diagnosis was first made from punch biopsies, following which the entire mass was excised.

Gross Pathology:
The specimen submitted was an approximately 7 x 7 cm elliptical section of haired skin, extending to panniculus musculature at its deep margin. The skin surface was centrally elevated and surrounding tissue compressed by a well-demarcated, firm, approximately 3-cm-diameter, multilobular mass. On cut surfaces, the mass was homogeneously white to pale pink, with lobules separated by septa of dense connective tissue.

Laboratory Results:
Serum biochemistry:
Within normal limits
CBC:

WBC 13.2x10^3/µL; 1% band neutrophils, 73% segmented neutrophils, 21% lymphocytes, 3% monocytes
HCT: 42.7%

Microscopic Description:
Haired skin: A densely cellular, multilobular, pseudoencapsulated, expansile neoplasm expands the subcutis, elevating the overlying dermis and compressing adjacent tissues. Neoplastic lobules sometimes abut or protrude into large vascular spaces, especially at the periphery, but remain separated from lumina by a thin layer of endothelium. The neoplastic cell population consists of round to polygonal to sometimes spindloid cells arranged in solid sheets, cords, or packets, supported by a fine fibrovascular stroma. The cells have moderate amounts of pale eosinophilic, vacuolated cytoplasm and indistinct cytoplasmic borders. Nuclei are centralized and round to ovoid, sometimes reniform or indented, with vesicular or dispersed chromatin. Anisocytosis and anisokaryosis are moderate. Mitotic figures are infrequent, with 4 mitotic figures seen in ten 400x fields in the most mitotically active regions. Scattered individual tumor cells are hypereosinophilic

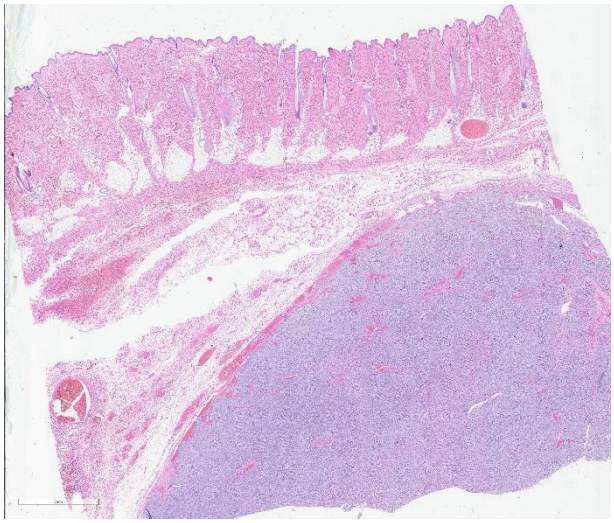

Figure 4-1. Haired skin, hyena. A single section of haired skin is submitted for examination. Within the subcutis, there is an expansile nodular neoplasm with a compression capsule and dilated blood vessels around it. (HE, 5X)

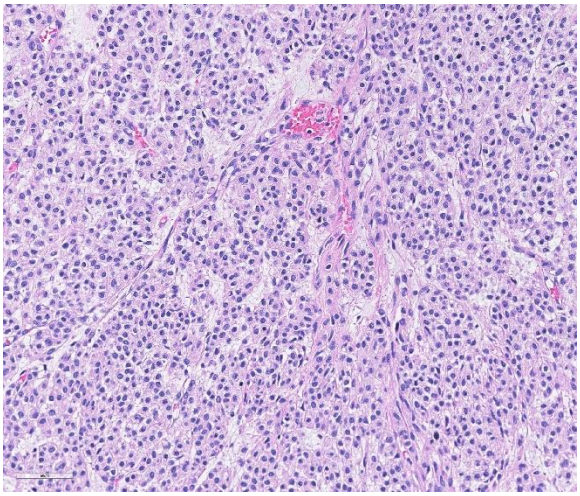

Figure 4-2. Haired skin, hyena. The neoplasm is composed of polygonal cells arranged in nests and packets. (HE, 215X)

and have condensed nuclear material, and there are a few small foci of necrosis and associated hemorrhage within the mass. In a few areas there are aggregates of fibrin and neutrophils adjacent to or partially occluding vascular lumina (thrombosis). In some sections, there is a broad zone of necrosis and regional hemorrhage with moderate numbers of hemosiderin- and hematoidin-laden macrophages (previous biopsy site).

Special stains and immunohistochemistry: Fine basement membranes surrounding individual tumor cells or small clusters of cells are immunoreactive for laminin and are also clearly highlighted with a Periodic acid-Schiff (PAS) stain. Neoplastic cells exhibit strong, punctate, cytoplasmic immunoreactivity for vimentin and α-smooth muscle actin. Immunohistochemical staining for desmin and pancytokeratin is negative.

Contributor's Morphologic Diagnoses:
Subcutis: glomus tumor

Contributor's Comment:
Glomus bodies are specialized arteriovenous shunts that function in thermoregulation by modifying capillary perfusion.[8] They are found most frequently in skin of the distal extremities, particularly in digital skin and

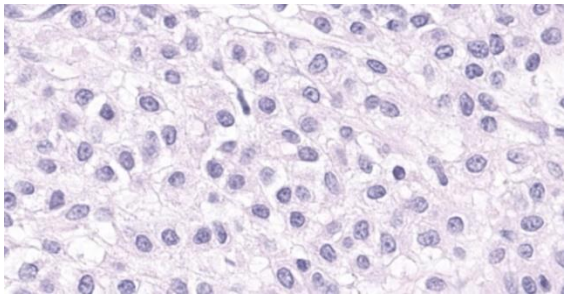

Figure 4-3. Haired skin, hyena. High magnification of neoplastic cells. (HE, 1025X)

subungual regions. In glomus bodies, the branching pre-glomic arterioles blend into arteriovenous anastomoses (Sucquet-Hoyer canals) with surrounding modified smooth muscle cells termed "glomus cells." They are richly invested with small nerves and blood vessels, and are circumscribed by dense, lamellar collagen.

Glomus tumors arise from the modified smooth muscle cells of the glomus body. They are uncommon but well-recognized in humans, where they occur most often as solitary nodules in subungual sites and in the dermis or subcutis of the digits and distal extremities.[8] They occur rarely in other sites, including visceral organs and bone.[8] Glomus tumors are often reported to be exquisitely painful, likely as a consequence of their rich peripheral nerve supply.[8]

Histologically, glomus tumors tend to be well-circumscribed and consist of densely cellular sheets and cords of epithelioid cells surrounding and abutting vascular structures, with fine basement membranes surrounding individual cells or clusters of cells. Several histologic variants are described in humans, including solid tumors, angiomatous glomus tumors (glomangiomas), myxoid glomus tumors, and oncocytic glomus tumors.[7] Malignant glomus tumors (glomangiosarcomas) are exceedingly rare. Even in glomus tumors with focal or regional cytologic features of malignancy, invasive behavior and distant metastases are uncommon.[7-9]

Using immunohistochemistry, the neoplastic cells are routinely positive for α-smooth muscle actin and vimentin. Desmin immunoreactivity is variable, and pancytokeratin immunoreactivity is uniformly negative. Basement membranes surrounding individual cells or clusters of cells can be demonstrated by PAS stains or by positive immunoreactivity for laminin and collagen IV.

Glomus tumors are rare in veterinary species, but have been reported in dogs, cats, horses, bovids, and non-human primates.[1-3,5,6,11,12,14,-16,18,20] Digital/subungual sites (or homologous regions) were affected in two dogs, a cat, and a horse.[2,3,5,20] Glomus tumors of the skin of the head or neck were reported in three equine cases, two of which were diagnosed as malignant variants.[2] In bovids, the two reports of glomus tumors describe neoplasms of the liver and of the urinary bladder.[10,16]

Proposed criteria suggesting malignancy include (1) large size (>2 cm diameter) with deep location (deep to muscular fascia); (2) presence of atypical mitotic figures; or (3) nuclear atypia with relatively high mitotic rate (>5/50 high power fields).[4] Vascular involvement is not reported to be associated with malignancy. In this case, the mass was

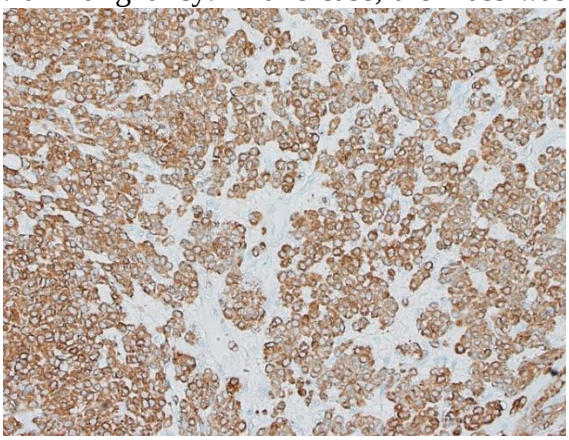

Figure 4-4. Haired skin, hyena. Neoplastic cells are strongly immunopositive for smooth muscle actin. (anti-SMA, 400X) (Photo courtesy of: Disease Investigations, San Diego Zoo Wildlife Alliance, http://institute.sandiegozoo.org/disease-investigations)

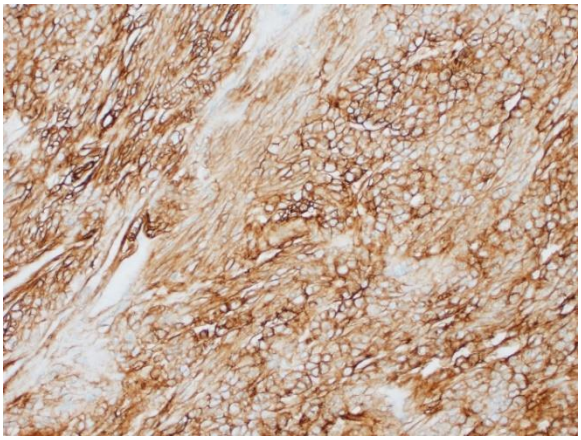

Figure 4-5. Haired skin, hyena. Neoplastic cells demonstrate strong membranous staining for laminin. (anti-laminin, 400X) (Photo courtesy of: Disease Investigations, San Diego Zoo Wildlife Alliance, http://institute.sandiegozoo.org/disease-investigations)

relatively large (~3 cm in diameter) but superficial. Atypical mitotic figures were not a significant feature. There was mild nuclear atypia, but a relatively high mitotic rate in some areas. Based on the criteria suggested above, this mass would warrant the (admittedly non-committal) designation of "glomus tumor of uncertain malignant potential." Radiographs taken at the time of excisional biopsy did not demonstrate metastatic lesions, and no evidence of recurrence or metastasis has been noted in the four months following excision.

Contributing Institution:
Disease Investigations
Institute for Conservation Research
San Diego Zoo Global
PO Box 120551
San Diego, CA 92112
http://institute.sandiegozoo.org/disease-investigations

JPC Diagnosis:
Haired skin: Glomus tumor.

JPC Comment:
This case is a classic example of a glomus tumor in a novel species. Glomus tumors are typically well-demarcated neoplasms composed of densely packed round to spindleloid cells which occasionally form nests.[10,17] Neoplastic cells frequently surround entrapped vascular channels, which may appear as blood-filled clefts, or may surround larger cavernous vessels (glomangiomas).[10,13] As the contributor mentions, the tumor is frequently associated with vessels and nerves, which may be visible along the tumor margin.[10] Differential diagnoses include Merkel cell tumors (arranged in a packet pattern), plasma cell tumors (cells are more pleiomorphic with eccentric nuclei), histocytomas (more top-heavy in distribution), other round cell neoplasms, and leiomyoma (particularly of the arrector pili muscle, or piloleiomyoma).[10] Other differentials considered by the moderator and participants were hidradenoma (as some areas of packeting mimicked tubules), melanoma, and a neuroendocrine tumor. When histologic findings are equivocal, special stains and IHCs may be necessary for differentiation, as described by the contributor.

A potential source for confusion in this uncommon veterinary neoplasm are the human entities glomus jugulotympanicum paraganglioma and glomus tympanicum paraganglioma.[13,19] While they contain the word glomus in the name, they are not associated with the glomus bodies in the dermis and subcutis. Rather, they are paragangliomas arising from neural-crest derived chemoreceptor cells of the paraganglia located along certain cranial nerves and within the jugular foramen.[13,19] As paragangliomas, neoplastic cells are positive for synapthophysin and chromogranin A on immunohistochemistry.[19]

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