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Department of Veterinary Pathology
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Conference Moderator:

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CASE I: S597-04 (AFIP 2937487).

Signalment: 18-month-old, male, roan antelope of west African origin, caprine (*Hippotragus equines koba*).

History: This animal was part of a juvenile bachelor herd (12 animals) on a 6,000 hectare game ranch in the South African lowveld, south of Kruger National Park. The herd was captured in Benin (west Africa) and translocated to this area in South Africa two months prior to release after a one month adaptation period under boma conditions. Approximately 16 days after release from the boma into the natural Acacia bushveld, this animal was noticed to exhibit depression. It was found dead the following day before therapeutic and clinical diagnostic procedures could be instituted.

Gross Pathology: A necropsy, external examination revealed excellent body condition. The tick load (mainly *Rhipicephalus evertsi* and *R. appendiculatus*) was considered moderate but acceptable, keeping in mind that a certain number of ticks would have detached from the carcass at death. The mucous membranes were severely icteric, and low viscosity (watery) of the blood on blood vessel incision was indicative of anemia. Generalized cortical lymphoid hyperplasia in all lymph nodes was noted. Upon evisceration, serosal petechiae and ecchymoses (almost sugillations in certain areas) were observed, especially on the rumen. Hemorrhages were also multifocally present in the paler renal cortices. The renal medulla showed marked bilirubin and hemoglobin staining and the urine was reddish (hemoglobinuria). Dipstick analysis revealed 2+ protein, 3+ bilirubin, 2+ hemoglobin/blood, and pH 6.5. The cut surface of the adrenal glands showed multiple cortical ecchymoses, without recognizable stress-induced cortical hypertrophy. The liver was dark purple-brown, had rounded edges, and there was a globally accentuated centrilobular/periportal pattern on cut surface. The gallbladder wall was severely thickened due to edema and extensive hemorrhage, with mild gallbladder distention. The content of the gallbladder was normal. The spleen was markedly enlarged (4-5 times normal size) and bulged on cut surface revealing a turgid pulpy consistency of the expanded red pulp. There were multiple petechiae and ecchymoses on the epicardial and endocardial surfaces. Yellow-white foam filled the tracheal lumen and extended for the whole of its length (pulmonary edema, severe). Macroscopic findings and blood smear results (see below) confirmed an etiological diagnosis of theileriosis.

Laboratory Results: Blood smear: Numerous (4+) blast-transformed lymphocytes with many, but not all, containing numerous intracytoplasmic theilerial macroschizonts (Koch's blue bodies); many active (vacuolated) monocytes with some containing phagocytosed parasitized erythrocytes; about 25% of the erythrocytes contained 1-4 theilerial merozoites (piroplasms); mild reticulocytosis; and severe thrombocytopenia.

Impression smears of the liver, spleen and lymph nodes revealed parasitized blast-transformed lymphocytes, similar to those found in the blood.

PCR for identification of Theileria species: Specimens from this case were evaluated by means of the reverse line blot (RLB) method and compared to all known *Theileria* and *Babesia* spp. Only a *Babesia/Theileria* catch-all signal was found. Sequencing of the protozoal DNA recovered from this case revealed a novel theilerial species which closely matched the protozoal DNA recovered from a Sable calf that died of theileriosis in 1992 in South Africa.⁷ This parasite has not been formally named, but *Theileria hippotragi* is the manuscript name.

Histopathologic Description: Liver: the most striking microscopic change in the liver is the accumulation of pleomorphic, lymphoblast-like round cells in the lumen of the sinusoids. These cells tend to occur as poorly-defined foci, especially in the vicinity of the portal triads. The hepatic cords are distorted and there is variable (pressure) atrophy of the hepatocytes. The larger round cells, many of which are bi- or multi-nucleate, often contain schizonts or large numbers of merozoites in their cytoplasm. Sinusoidal congestion is apparent, especially around the central veins; this is accompanied by cholestasis, evidenced by markedly distended bile canaliculi.

Contributor's Morphologic Diagnosis: Liver, lymphoblastic sinusoidal infiltration, with architectural disruption, congestion, cholestasis, roan antelope.

Contributor's Comment: Theileriasis in African wildlife, previously known as cytauxzoonosis, has been reported in eland (*Taurotragus oryx*), common duiker (*Sylvicapra grimmia*), greater kudu (*Tragelaphus strepticeos*), giraffe (*Girrafa camelopardis*), roan antelope (*Hippotragus equinus*), sable antelope (*Hippotragus niger*), and tsessebe (*Damaliscus lunatus*). The morphological pathology is only reasonably well-documented in eland,² grey duiker,⁵ giraffe,⁴ and tsessebe.³ Both the schizont and piroplasms stages appear to be pathogenic. Schizogony occurs in lymphocytes and/or monocytes/macrophages (the identity of the host cell type is still contentious). Parasite-transformed host cells tend to accumulate in capillary and sinusoidal beds, especially those of the liver, spleen, lymph nodes, lungs and kidneys (particularly the glomeruli). Microscopic foci of necrosis and petechial hemorrhages in these organs (not a marked feature in the liver presented here) are most likely a consequence of circulatory disturbances in these vascular beds. Piroplasms inhabit red blood cells, where they multiply by binary fission. Presumed piroplasm-induced injury to red blood cells varies in severity, both within and between affected wildlife species. In some cases, severe anemia and icterus are apparent.

In South Africa, theileriasis of wildlife appears to have a significant impact on free-living populations of roan, sable, and tsessebe. In certain habitats and circumstances the disease is responsible for high morbidity and high mortality rates in juveniles; this results in low recruitment rates and extirpation of metapopulations. Research into control strategies for the disease in these three valuable antelope species is currently being conducted.

Theileria-like piroplasms and/or schizonts have been found in both asymptomatic and sick Afrotropical wildlife species belonging to a number of different orders and many families. The systematic and taxonomy of this, probably large, clade of protozoal organisms is in its infancy, but the use of nucleotide-sequencing techniques will certainly provide much needed impetus for the required research.

AFIP Diagnosis: Liver: Hepatitis, lymphoblastic, diffuse, marked, with bile stasis; multifocal vasculitis; hepatocellular degeneration and necrosis; multifocal hemorrhage; and many lymphoid intracytoplasmic protozoal schizonts.

Conference Comment: There was intensive discussion as to the most appropriate classification of the liver lesion among conference attendees; some participants favored hepatitis, while others favored the diagnosis of lymphocytosis. In the opinion of the moderator, when the hepatic parenchyma and/or sinusoids are infiltrated by inflammatory cells the most appropriate morphologic diagnosis is hepatitis. In contrast, others favored the diagnosis of lymphocytosis/lymphoblastosis based on the pathogenesis described in the literature, and preferred to classify the remaining hepatic changes as secondary. Two reference texts define acute hepatitis as having morphologic characteristics of a combination of inflammation and hepatocellular apoptosis and necrosis; regeneration may or may not be present.^{1,8} A third text defines hepatitis as being either focal or diffuse infectious processes or leukocyte inflammatory infiltrate regardless of cause.⁹ Based on either set of criteria, there is sufficient hepatocellular necrosis and leukocytic (lymphoblastic lymphocytic) infiltration to characterized the lesion as hepatitis.

The life cycle of theilerial organisms was reviewed. Briefly, the organism is transmitted through the bite of *Rhipicephalus* and *Hyalomma* species ticks. Once inside the host, the sporozoites infect lymphocytes and induce transformation to lymphoblasts. Macroschizonts, found in the cytoplasm of lymphoblasts, are referred to as Koch's blue bodies. The macroschizonts proceed to the microschant stage, causing cell lysis and release of merozoites which subsequently infect erythrocytes. Once inside the erythrocytes, they enter the final stage forming piroplasms.⁹

The clinical signs associated with theileriosis begin with high fever and diffuse lymphadenopathy around two weeks post-infection. Dyspnea, progressive anemia, and lymphocytolysis contribute to the acute form of the disease and subsequent death of the animal. Gross findings include diffuse enlargement of lymphoid tissues, serous effusions, ulcerative abomasitis, an enlarged spleen early in disease which later becomes shrunken, mottled gray-white patches

in the liver and kidney, and congested, edematous lungs. Histologically, there is variation in the lesions based on the progression of the disease process. Initially there is diffuse lymphoid hyperplasia, lymphocytolysis of small lymphocytes with replacement by lymphoblastic lymphocytes, interstitial infiltration of the kidney, and lymphocyte infiltration of pulmonary interstitium resulting in alveolitis. Later in the disease process, lymphoid tissues are shrunken with hemorrhage and fibrin throughout the parenchyma and the bone marrow is hypocellular.⁹

Finally, conference participants briefly discussed the difference between theileriosis and cytauxzoonosis; while theilerial protozoa infect lymphocytes, cytauxzoons infect macrophages.⁹

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CASE II: AR05-406 (AFIP 3026271).

Signalment: Adult, gender unknown, northern leopard frog, amphibian (*Rana pipiens*).

History: The animal was found dead after a clinical history of progressive abdominal distention of several weeks' duration.

Gross Pathology: The coelomic cavity was distended by 2-3 mL of serosanguineous, clear fluid. The kidneys were unable to be identified grossly, having been distorted and replaced by a 0.5 x 0.5 cm white, nodular, firm mass.

Histopathologic Description: Kidney: Remnants of normal renal architecture consisting of rare glomeruli and tubules are found within an invasive multilobular, non-encapsulated mass of epithelial cells which form irregular glandular structures separated by collagenous stroma. The epithelial cells are cuboidal to columnar with moderate amounts of eosinophilic cytoplasm and centrally located 8-10 micron diameter nuclei with dispersed, coarsely stippled chromatin. Rare mitoses are noted. The lumina of the glandular structures often contain cell debris and eosinophilic homogenous material (protein).

Contributor's Morphologic Diagnosis: Renal adenocarcinoma

Contributor's Comment: Lucké's renal adenocarcinoma is an invasive and malignant tumor spontaneously affecting the northern leopard frog (*Rana pipiens*) and caused by Ranid Herpesvirus-1, a currently unclassified herpesvirus. Viral morphology is icosahedral, with virions measuring 95-110 nm in diameter. Infected leopard frogs are mainly found in the northeastern and north central United States. Affected frogs may not show signs until

advanced tumor growth, with emaciation, lethargy, ascites and sudden death occurring most commonly. Tumor growth can affect one or both kidneys, and grossly the tumors are pale tan and multilobulated. Histologically papillary adenocarcinomas are the most common morphology.⁹

Embryos and larvae are susceptible to viral infection but tumor growth is not recognized until young adulthood.¹⁰ There is a seasonal change in tumor prevalence, with tumors being most common in early spring when frogs emerge from hibernation. The virus replicates during cooler winter temperatures and eosinophilic intranuclear inclusions and virions can be detected during this replication period. Virions are not detected in tumors maintained at warm temperatures;^{11,12} however, during this latent period the RHV-1 genome is present.¹ Increased tumor invasiveness and metastasis has also been shown to be temperature dependent. Tumor metastasis occurs frequently in warm temperatures (77%) and is not recognized in cold temperatures. Temperature-dependent tumor collagenase activity has been demonstrated and is believed to contribute to the differential tumor growth and metastasis.^{6,8}

AFIP Diagnosis: Kidney: Renal adenocarcinoma, papillary.

Conference Comment: Conference participants discussed other oncogenic herpesviruses. In humans, Epstein-Barr virus is associated with Burkitt's lymphosarcoma in Africa, and human herpesvirus 8 causes Kaposi's sarcoma, most commonly in AIDS patients. The following chart, adapted from *Fenner's Veterinary Virology*, outlines some of the oncogenic herpesviruses of interest to veterinary species.^{3,4,5,7}

Virus Family/Genus	Virus	Tumor Type Induced
<i>Herpesviridae / Alphaherpesvirinae / Mardivirus</i>	Marek's disease virus (Gallid herpesvirus-2)	T-cell lymphosarcoma in chickens
<i>Herpesviridae / Gammaherpesvirinae / Rhadinovirus</i>	Ateline herpesvirus-2 and saimirine herpesvirus-2	Lymphoma and leukemia in aberrant hosts
<i>Herpesviridae / Gammaherpesvirinae / Lymphocryptovirus</i>	Epstein-Barr virus	Burkitt's lymphoma, nasopharyngeal carcinoma and B-cell lymphomas in humans and non-human primates
	Baboon herpesvirus (Papiine herpesvirus-1)	Lymphoma in baboons
<i>Herpesviridae / Gammaherpesvirinae / Rhadinovirus</i>	Cottontail rabbit herpesvirus	Lymphoma in rabbits
<i>Alloherpesviridae / ranid herpesvirus</i>	Lucké frog herpesvirus (Ranid herpesvirus-1)	Renal adenocarcinoma in frogs
<i>Herpesviridae / Gammaherpesvirinae</i>	Otarine herpesvirus-1	Urogenital carcinoma in California sea lions
<i>Herpesviridae / Alphaherpesvirinae</i>	Psittacid herpesvirus-1	Cloacal and crop papillomas in parrots (Internal papillomatosis of parrots); associated with pancreatic duct carcinoma in a macaw

Since the submission of this case, Ranid herpesvirus-1 has been classified as a member of the *Alloherpesviridae* family, *Batrachovirus* in the International Committee on Virus Taxonomy's most recent release.²

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http://www.wfubmc.edu/schoolOfMedicine/schoolOfMedicine_default.aspx?id=26651

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CASE III: BERNE 2/10 (AFIP 3164904).

Signalment: Adult, female, Chagoi Koi carp, piscine (*Cyprinus carpio*).

History: The affected animal originated out of a group of koi carp of different age classes and strains kept in a garden pond. About 10 animals, Kigo and Chagoi, were affected showing singular to several small, up to 1 cm in diameter, nodules on various locations on the skin.

Gross Pathology: Affected skin tissue was submitted for histopathological examination (surgical biopsy). Small nodular swellings up to 1 cm diameter were recorded.

Histopathologic Description: Skin: The dermis is markedly expanded by severe edema, small amounts of macrophages (with vacuolation of the cytoplasm, foamy macrophages), lymphocytes, plasma cells and scattered neutrophils and eosinophilic granular cells and by multiple numerous, round to elongated, different sized (50µm to 200 µm) cystic-like structures comprising a thick-wall (approximately 10µm) hyaline capsule filled with basophilic round structures of varying size (3 µm to 10 µm), interpreted as *Dermocystidium* spores. The spores contain a large amount of eosinophilic cytoplasm with a central hyaline refractile body and a peripheral small dark nucleus. In several cysts the organisms are degenerated. There is moderate edema within the epidermis (spongiosis).

Contributor's Morphologic Diagnosis: Dermatitis, lymphohistiocytic, moderate, diffuse, chronic with severe edema and intralesional *Dermocystidium* cysts.

Contributor's Comment: The genus *Dermocystidium* are unicellular parasites of an uncertain taxonomic classification³ that has been reported as a yet-unnamed clade of eukaryotic protistan organisms in aquatic animals like fish (*Dermocystidium*, *Ichthyophonus*, rosette agents), amphibians (*Dermocystidium*), and crustaceans (*Psorospermium*).⁶ More than 20 species of *Dermocystidium* were found as cysts in skin or gill, or as systemic infections in carp, goldfish, salmonids, eels, newts and frogs (Table 1).¹ *Dermocystidium percae* infection in perch (*Perca fluviatilis*) has a high prevalence in polluted environments which can act as an important stress factor.⁵ *Dermocystidium koi* produces nodular swellings up to 1 cm in diameter in the skin of koi (*Cyprinus caprio*). Minimal local inflammation and edema is observed at the edge of the swellings. The fungal nature of *D. koi* suggests hyphae and spore development which microscopically have been described as aseptate hyphae.^{4,8} There is little information about the epidemiology and life cycle of *D. koi*, but it is presumed to occur worldwide. *Dermocystidium salmonis* is a more significant pathogen, resulting in extensive gill pathology and high mortality.³

AFIP Diagnosis: Skin: Dermatitis, lymphohistiocytic and granulocytic, diffuse, mild with moderate edema and many parasitic cysts.

Conference Comment: Conference participants commented on slide variation, with some sections having epidermal parasitic cysts. Occasionally, these epidermal cysts showed evidence of rupture and exfoliation of parasitic spores.

In the opinion of the moderator the dermal edema is the most striking histologic lesion in this case. Conference participants speculated on the pathogenesis for the edema, and three theories were briefly discussed: disruption of the epidermis resulting in loss of osmoregulation; primary cutaneous vasculitis; or secondary, bystander damage to blood vessels by the dermal inflammatory infiltrates.

As the contributor notes, *Dermocystidium* spp. often cause gill lesions in addition to dermal lesions. The disease in fry is particularly devastating, resulting in death due to anoxia from massive gill infection which physically prevents the operculum from closing.⁷ In addition to the cutaneous lesions seen in this case, other histologic findings with *Dermocystidium* spp. infection include granulomatous inflammation of gills with apoptosis and hyperplasia of the lamellar epithelium, and splenic congestion and fibrosis surrounding cysts.⁷ The gross lesions of *Dermocystidium* spp. infection in the skin are striking, presenting as numerous 1 mm linear white streaks that closely resemble infection with *Epitheliocystis* spp. The differential diagnosis for protozoal skin and gill lesions in fishes includes amoebae, coccidia, microsporidia, myxosporidia (*Myxobolus* spp.), *Ichthyophonus* spp., trichodinids, *Chilodonella* spp., and *Ichthyobodo* spp.²

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<http://www.itpa.vetsuisse.unibe.ch/html>

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CASE IV: 53079 (AFIP 3164991).

Signalment: 20-year-old, male, shingleback skink, reptile (*Tiliqua rugosus*).

History: This skink presented for slowly progressive weight loss. A mass was seen in the oral cavity during clinical examination. Examination of cytology and biopsy specimens revealed narrow-based budding yeasts consistent with *Cryptococcus* sp. *Cryptococcus neoformans* type A/D was cultured from the mass. The skink was treated long term with antifungal medications, and at a follow-up examination two months later the oral lesion had resolved. However, despite ongoing antifungal treatments, the skink never recovered completely and periodically needed assisted feeding to maintain weight. Nine months after initial presentation the skink's condition declined and it died.

Gross Pathology: The animal was 496 g and in good body condition with adequate adipose stores. Disseminated throughout the lungs, but more concentrated cranially, were approximately 10-20, multifocal to coalescing, soft, pale tan nodules ranging from 0.3 to 0.6 cm in diameter. The nodules bulged into both the coelomic and luminal aspects of the lung and were homogeneously pale tan and gelatinous on section.

Laboratory Results: *Cryptococcus neoformans* type A/D was cultured from an oral cavity mass 9 months prior to death.

Histopathologic Description: Lung: Expanding the parenchyma are multiple nodules composed of sheets of foamy macrophages that sometimes occlude faveolar openings. Large numbers of yeast organisms are present in these areas predominantly within macrophages and occasionally extracellularly in small sheets and clusters. The yeasts are characterized by a central round, pale-staining, refractile, amphophilic to basophilic, 4-12 um diameter yeast body surrounded by a 2-6 um clear space (capsule). The yeasts frequently exhibit narrow-based budding. Small numbers of lymphocytes, plasma cells and multinucleated giant cells are also present. Adjacent faveoli are frequently dilated and filled with wispy to hyaline eosinophilic material mixed with variable numbers of similar yeasts and a small amount of necrotic cellular and mineralized debris (some variation between slides).

Brain, cerebrum: Throughout the section, the meninges are markedly expanded by large numbers of foamy macrophages and similar yeasts that compress and multifocally extend into the neuroparenchyma along blood vessels (some variation between slides). Small numbers of lymphocytes, plasma cells, and multinucleated giant cells are also present.

Contributor's Morphologic Diagnosis: 1. Lung: moderate chronic multifocal histiocytic pneumonia with intralesional yeasts (etiology: *Cryptococcus neoformans*)
2. Brain: severe chronic diffuse histiocytic meningoencephalitis with intralesional yeasts (etiology: *Cryptococcus neoformans*)
3. Oral cavity (not submitted): moderate focal histiocytic stomatitis with intralesional yeasts (etiology: *Cryptococcus neoformans*)

Contributor's Comment: *Cryptococcus neoformans* is a basidiomycete yeast-like fungus with a global distribution. It can cause disease in a variety of species and is the most common cause of systemic fungal disease in domestic cats.² There are 2 varieties of *C. neoformans*: *neoformans* and *gattii*, with four major serotypes, A B C D. Isolates of *C. neoformans* (formerly *C. neoformans* var. *neoformans*) have capsular serotypes A or D or both (AD), and isolates of *C. gattii* (formerly *C. neoformans* var. *gattii*) have serotypes B and C.^{2,7} *Cryptococcus neoformans* is found in soil, bird feces, and decaying organic matter. *Cryptococcus gattii* has been found in association with eucalyptus trees, bat guano and decaying wood.^{2,9}

Grossly, lesions may be gelatinous or solid masses, or ulcerated nodules. In histologic sections, the organisms are easily identified by their characteristic size (2-20 microns), narrow-based budding and thick capsule that can be highlighted with a mucicarmine stain.³ At low magnification, the lesions have a vacuolated or bubbly appearance due to the thick yeast capsule. Typically, inflammatory cells are present in low numbers and are primarily epithelioid macrophages and fewer lymphocytes and plasma cells.²

Cryptococcosis is only rarely seen in reptiles and amphibians. It has been reported in a common anaconda (*Eunectes murinus*),⁶ an eastern water skink (*Eulamprus quoyii*),⁴ and a toad.⁸ Recently *C. gattii* has been in the news as an emerging pathogen of animals and humans in the northwestern United States and Vancouver, Canada.^{1,9}

This is the first instance of cryptococcosis in a reptile at our institution. The lesions in this skink have a similar appearance and distribution as those reported for other species. The inflammation in the oral cavity was not grossly visible, and in histologic sections the inflammation did not extend above the mucosal surface. We have been unable to determine whether the oral cavity mass noted nine months prior to death was the initial site of infection, with later spread to lung and brain, or whether systemic infection was already present at that time. There was no evidence that this skink had a compromised immune system.

AFIP Diagnosis: 1. Lung: Pneumonia, histiocytic, nodular, multifocal, marked, with many narrow-based budding encapsulated yeasts, etiology consistent with *Cryptococcus* spp.
2. Brain: Meningitis, histiocytic, diffuse, marked with many narrow-based budding encapsulated yeasts, etiology consistent with *Cryptococcus* spp.

Conference Comment: Several conference participants classified the lesions as granulomatous; for a review of the distinction between histiocytic and granulomatous inflammation, please see WSC 2010 Conference 3, Case II.

Conference participants also discussed the various virulence factors of *Cryptococcus neoformans*, which include a polysaccharide capsule, melanin production and several enzymes. The polysaccharide capsule not only prevents phagocytosis by host immune cells, it also inhibits inflammatory cell migration and recruitment; activates complement; and suppresses T-cell response.^{2,5} The capsule also undergoes phenotype switching, changing the

capsule structure and size and allowing it to further elude the immune system.⁵ Melanin production is believed to contribute to virulence by acting as an antioxidant to counteract reactive oxygen and nitrogen species produced by the host. Finally, the yeast secretes several enzymes, one of which is serine proteinase, which cleaves fibronectin and basement membrane proteins to allow tissue invasion; other secreted factors modulate the host immune response.⁵ Participants also discussed the differences between *C. neoformans* and *C. gattii*; while the former typically affects immunocompromised individuals, the latter is able to cause disease in healthy, immunocompetent individuals.

Immunity to *C. neoformans* depends on delayed-type hypersensitivity reaction. Briefly, interferon-gamma (IFN- γ) and other cytokines recruit and activate macrophages, and possibly neutrophils, resulting in production of reactive oxygen and nitrogen species. Additionally, cytotoxic T-cell response may limit infection through direct response to the yeast.²

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