The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
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Conference 23

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Conference Moderator:

Dr. Don Nichols, DVM, Diplomate ACVP

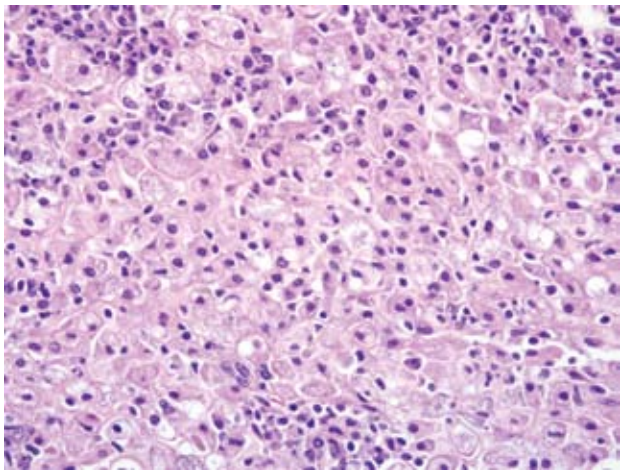
CASE I – 060536-20 (AFIP 3113832)

Signalment: Adult male African green monkey (*Chlorocebus aethiops*). All monkeys described in this report were maintained in a facility at the US Army Medical Research Institute of Infectious Diseases accredited by the American Association for Accreditation of Laboratory Animal Care. All research was conducted under approved animal protocols in adherence with the Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, National Institutes of Health Publication No. 86–23, revised 1996).

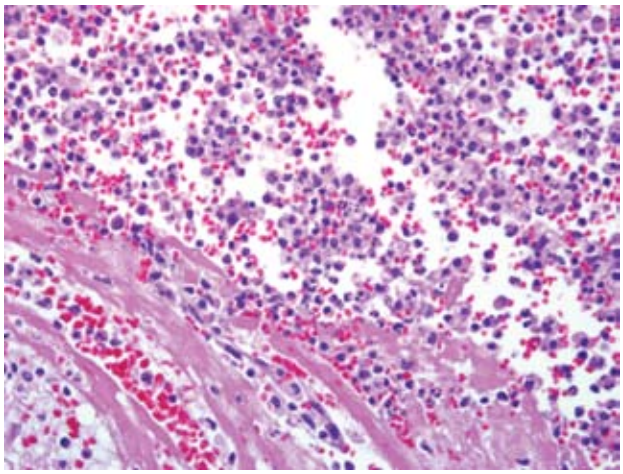
History: The African green monkey (AGM) was received at our facility on 13 June, 2003 and in good condition. ITS telemetry surgery was performed on 1 March 2006 and the AGM recovered without incident. The next month, on 18 April 2006, prior to being placed into biocontainment, a routine physical examination was performed. An 8- to 10-cm-diameter mass was found on the right dorsal flank. No other abnormal findings were observed at the time. Within 24 hours of initial discovery, the fluid-filled mass ruptured and was draining a thick, mucoid discharge. The abscess was cultured, surgically debrided and lavaged, a Penrose drain was placed, and antibiotic treatment was initiated. An abdominal mass was palpated 24 hours following surgery and was described as a 3 cm by 1 cm irregularly shaped, firm mass in the cranial abdomen. The mass enlarged over several days, and exploratory laparotomy revealed mesenteric abscesses centered at the ileocecocolic junction and also involving the duodenum, ileum, cecum, colon, the right ureter, and the body wall inferior to the right kidney. Surgical removal was not possible, and the animal was euthanatized.

Gross Pathology: The most significant findings at necropsy were chronic pyogranulomatous peritonitis and polyserositis; chronic pyogranulomatous panniculitis of the right flank (surgical abscess site); and a defect in the abdominal wall subjacent to the panniculitis. There were multiple fibrous adhesions present in the cranial abdomen. Buried within these adhesions were multiple chronic abscesses that sometimes contained a viscous fluid. Serosal and mesenteric adhesions enveloped and intertwined loops of small and large bowel, adhered to the right ureter and kidney, and extended into the retroperitoneal space with fingerlike projections. The abdominal wall inferior to the subcutaneous lesion of the right flank was disrupted, or breached, and an abdominal fibrous band of tissue was present extending to the cecum.

***Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists, and the C. L. Davis Foundation.**



1-1. Mesentery, African Green Monkey. Expanding the mesentery is a cellular infiltrate composed of numerous epithelioid macrophages with abundant foamy vacuolated cytoplasm admixed with fewer neutrophils, lymphocytes and plasma cells. (HE 400X)



1-2. Lymph node, African Green Monkey. Effacing normal nodal architecture are large swaths of fibrin, necrosis, pyogranulomatous cellular infiltrate, and abundant hemorrhage. (HE 400X)

Laboratory Results: Bacteria in the abscess fluid were identified as *Klebsiella pneumoniae* by routine culture and biochemical methods and were further characterized as *K. pneumoniae* subsp. pneumoniae serotype K2 by capsular serotyping using a slide agglutination technique.[1] Furthermore, bacterial colonies plated on a blood agar plate exhibited a hypermucoviscosity phenotype, as demonstrated by a positive string test (formation of a 0.5 mm mucoviscous string when a loop is passed through a colony). Genetic analysis of the sequence of a 760-bp amplicon of the bacterial 16S rRNA from the isolate was submitted to a basic local alignment search tool search and found to be 99% identical to several *K. pneumoniae* entries. Additional analysis for 2 novel genes, magA (mucoviscosity-associated gene A) and rmpA gene (regulator of the mucoid phenotype) suggested as potential hypermucoviscosity-associated genes in human cases, was performed by PCR using previously described primers.[8] The bacterial isolate from this case was positive for the rmpA gene and was negative for the magA gene.[6]

Histopathologic Description: Diffusely, the serosa of the small intestine (ileum at ileocecocolic junction) and mesentery are greatly expanded by broad bands of fibrous tissue that strongly adhere mesentery and serosa into a mass. There are mesenteric lymph nodes surrounded and entrapped by fibrous tissue. Interspersed sporadically within the fibrous tissue are abscesses composed primarily of macrophages that contain abundant cytoplasm, fewer lymphocytes and plasma cells, rare neutrophils, cellular debris, and sometimes hemorrhage **(Fig. 1-1)**.

Select mesenteric lymph nodes are multifocally expanded and disrupted by large numbers of macrophages that contain abundant cytoplasm, cellular and karyorrhectic debris, edema, and hemorrhage **(Fig. 1-2)**.

The mucosa of the small intestine is multifocally eroded. Occasionally cellular debris may be found in the lumen and in the submucosa. Lymphocytes and plasma cells mildly expand the submucosa. There are moderate numbers of bacilli multifocally within the lumen, on the surface of erosive areas, and invading the mucosa and submucosa.

Contributor's Morphologic Diagnosis:

1. Mesentery and ileum: Peritonitis and serositis, histiocytic, chronic, multifocal, marked, with fibrosis, necrosis, and rare hemorrhage, African green monkey (*Chlorocebus aethiops*)

2. Lymph node, mesenteric: Lymphadenitis, histiocytic, subacute, multifocal, moderate, with necrosis and hemorrhage

3. Small Intestine: Enteritis, erosive, multifocal, mild to moderate, with bacilli

Contributor's Comment: Within a year and 5 months, six additional AGM with clinical and pathologic features similar to the AGM described here, were positively diagnosed with *K. pneumoniae* by bacterial culture and/or immunohistochemistry. Clinical signs in these additional monkeys varied from none to palpable abdominal masses

noted during routine clinical examination. One monkey was found dead in its cage with no premonitory clinical signs. All seven affected monkeys were either housed within, or were in contact with monkeys housed within, one animal room in our facility.

Klebsiella pneumoniae is a gram-negative, aerobic, nonmotile bacillus and is a common cause of a wide range of infections in humans and animals.[6] Our differential diagnosis for Gram-negative pathogens in nonhuman primates included, but were not limited to, *Yersinia enterocolitica* (forms large colonies in tissues, which were not present in our cases), *Shigella* sp. (*S. dysenteriae, S. Flexneri, S. boydii, S. sonnei*), *Campylobacter* sp. *(C. jejuni, C. coli), Salmonella* sp. *(S. typhimurium, S. Dublin, S. enteriditis, S. Stanley*). In Old and New World monkeys, infection with *K. pneumoniae* causes pneumonia, meningitis, peritonitis, cystitis, and septicemia.[9] *K. pneumoniae* also constitutes normal fecal and oral flora in many nonhuman primates. In the past two decades, a new type of invasive *K. pneumoniae* disease has emerged in humans in Taiwan and other Asian countries, and more recently from non-Asian countries, including the USA.[3,4,5,7] Fatal human infections with invasive strains of *K. pneumoniae* involve pulmonary emboli or abscess, meningitis, endophthalmitis, osteomyelitis, or brain abscess. Recently, a highly invasive *K. pneumoniae* causing primary liver abscesses in humans has also been reported.[3] These invasive, abscess-forming strains of *K. pneumoniae* are associated with the so-called hypermucoviscosity (HMV) phenotype, a bacterial colony trait identified by a positive string test.[4,5,6] The HMV phenotype is seen in *K. pneumoniae* expressing either the capsular serotypes K1 or K2. K1 serotypes of *K. pneumoniae* have two potentially important genes, rmpA, a transcriptional activator of colanic acid biosynthesis, [10] and magA, which encodes a 43-kD outer membrane protein. Five K2 serotypes of *K. pneumoniae* also have rmpA but do not have magA. Capsular serotypes K1 and K2 are reported to play an important role in the invasive ability of HMV *K. pneumoniae*.[3,10] The role of rmpA and magA in the pathogenesis of invasive *K. pneumoniae*, however, seems less certain. *K. pneumoniae* expressing the HMV phenotype has not been reported to cause natural disease in nonhuman primates, nor in other animal species.

The means by which the causative *K. pneumoniae* may have spread or caused disease in individual monkeys in our colony is unknown. The only significant epidemiologic factor we identified was that affected monkeys were maintained in the same room or had contact with a monkey housed in that room. African green monkeys may be quite susceptible to invasive *K. pneumoniae* infection. Therefore, veterinarians, laboratory workers, and research pathologists should be aware of this pathogen as a cause of abdominal masses and multisystemic abscessation in the AGM. In addition, the AGM may provide another useful animal model to understand the pathogenesis of this emerging human pathogen.

AFIP Diagnosis: 1. Ileocecocolic junction: Serositis and peritonitis, granulomatous, multifocal to coalescing, severe with marked fibrosis
2. Lymph node, mesenteric: Lymphadenitis, pyogranulomatous, diffuse, severe

Conference Comment: The abundant foamy material within macrophages was discussed during the conference. Dr. Nichols speculated that the foamy material within macrophages may be phagocytized mucus which the *Klebseilla* bacteria have produced. He also mentioned it is important to rule out mycobacterial causes when macrophages of this type are present in a lesion. In this case, an acid-fast stain was done and was negative.

In domestic animals, *Klesiella pneumonia* is the cause of numerous maladies. In foals, *K. pneumoniae* is a common cause of neonatal septicemia and pneumonia.[1,2] *K. pneumoniae* has also been commonly implicated in equine abortions.[8]

Contributing Institution: USAMRIID, Pathology Division, 1425 Porter Street, Ft. Detrick, MD

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CASE II – L08-2351 (AFIP 3103348)

Signalment: Adult, female, South African-clawed frogs (*Xenopus laevis*)

History: This frog belonged to a colony of female South African-clawed frogs, the ova of which were used for cell-cycle research purposes. The frogs were obtained from a commercial vendor and were originally laboratory-bred. The investigator had reported "red leg," lethargy, and sudden unexpected mortalities of a few of the frogs. This was one of 5 frogs submitted dead (and frozen) for pathologic investigation.

Gross Pathology: This 135 g female South African-clawed frog presented dead in lean body condition and good but frozen post mortem condition (mild autolysis). There was marked erythema of the entire ventral surface of the frog, and diffusely, the skin easily sloughed off. All other organs and tissues were within normal gross limits.

Laboratory Results: No growth was obtained from bacterial cultures of the skin and heart blood from all 5 frogs.

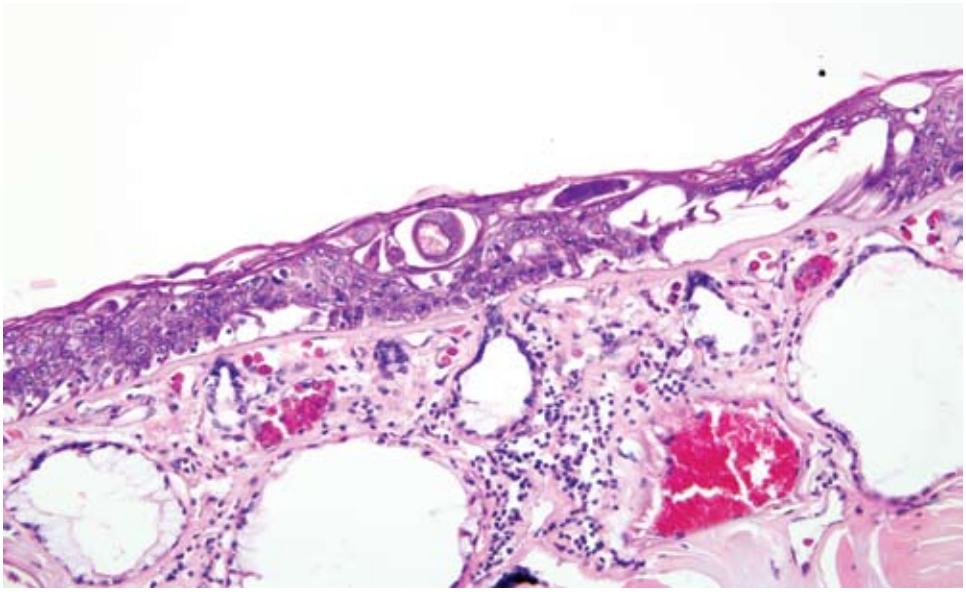
Histopathologic Description: Sections of skin are examined, revealing marked epidermal hyperplasia (characterized predominantly by acanthosis) associated with presence of cross-, tangential, and longitudinal sections of aphasmid adult nematodes that lie within tortuous intraepidermal tunnels **(Fig. 2-1)**. The aphasmid adult nematodes are approximately 25 to 50 microns in diameter, and possess a thin smooth external cuticle with hypodermal bacillary bands and unapparent musculature that surround a pseudocoelom that may contain an esophagus encased by a stichosome, and a single often gravid uterus. The eggs are roughly ovoid, approximately 25 to 35 microns in diameter, and may or may not be embryonated. Additionally within the tunnels (with or without associations with the adult nematodes), small numbers of embryonated eggs, small numbers of heterophils, small amounts of amorphous eosinophilic cellular debris, and small colonies of basophilic coccobacilli are seen. There is also separation of epidermis from underlying dermis with or without complete loss of overlying epidermis. There is a mild, multifocal, heterophilic and lymphocytic infiltrate in the underlying stratum spongiosum, and superficial vessels within the stratum spongiosum are distended with moderate numbers of heterophils.

Contributor's Morphologic Diagnosis: 1. Skin, epidermal hyperplasia, diffuse, marked, chronic with mild heterophilic and lymphocytic chronic-active dermatitis, and intraepidermal adult aphasmid nematodes and embryonated eggs (consistent with *Capillaria xenopodis*/*Pseudocapillaria xenopi*)

Contributor's Comment: All 5 South African-clawed frogs examined from this investigation were infected with *Capillaria xenopodis*/*Pseudocapillaroides xenopi*, which is an aphasmid nematode.

In general, the key histologic characteristics of aphasmid nematodes include a thin smooth external cuticle with hypodermal bacillary bands, unapparent musculature, an esophagus encased by a stichosome, and presence of a single uterus in females.[1] Additional specific microscopic features of *Capillaria xenopodis*/*Pseudocapillaroides xenopi* include sexual dimorphism (females are 4 times longer and 2 times wider than males), and the presence of unembryonated and embryonated eggs within the uterus (as opposed to presence of only unembryonated eggs in other aphasmid nematodes).[4]

Capillaria xenopodis/*Pseudocapillaroides xenopi* is the only reported nematode parasite of the epidermis of South African-clawed frogs, and is potentially a significant cause of morbidity and mortality in populations of South African-clawed frogs in the laboratory setting. Clinically, a small proportion of infected frogs in a laboratory animal population will display signs of *Capillaria xenopodis*/*Pseudocapillaroides xenopi* infection, which is often characterized as a nonspecific "wasting syndrome" (often lasting 3 to 4 months) that may include one or more of



2-1. Skin, Xenopus frog. Diffuse epidermal hyperplasia up to two times normal thickness and numerous cross and tangential sections of adult and larval nematodes. (HE 400X)

the following signs: lethargy, anorexia, skin color change, rough "flaky" skin, decreased egg production in females, and unexpected death. Often the first sign of this syndrome is the presence of large fragments/"flakes" of desquamated epithelium in the tank water.[3]

Histologically, *Capillaria xenopodis*/*Pseudocapillaroides xenopi* infection is characterized by profound epidermal hyperplasia (and variable associated epidermal/dermal inflammation) along with the presence of the nematodes and eggs in tunnels within the epidermis. This epidermal hyperplasia is thought to cause significant impairment of normal epidermal functions that result in overall debilitation and/or death of the animal. Specifically, the normal respiratory and metabolic functions (including gas exchange, waste removal, and osmotic balance) of the skin are compromised. Additionally, the normal physical barrier of the skin is compromised, often allowing for localized secondary skin infections and septicemia (including infection by Gram-negative bacteria, such as *Aeromonas hydrophila*, the cause of "red leg").[3]

Diagnosis of infection can be made by direct examination of unstained skin sections (including the desquamated "flakes" in the tank water), and histopathology.

Capillaria xenopodis/*Pseudocapillaroides xenopi* has a direct lifecycle, with all stages of the lifecycle completed within the epidermis of the skin. Because of the direct lifecycle, autoinfection is also possible.[3] Levamisole has been described as an effective treatment for *Capillaria xenopodis*/*Pseudocapillaroides xenopi* infection in laboratory South African-clawed frogs.[2]

Other aphasmid parasites that primarily infect and cause hyperplasia of epidermal/epithelial surfaces include: *Capillaria* spp. (*C. annulata, C. contorta, C. obsignata*) in the crop and esophagus of birds; *Capillaria philippinensis* in the small intestines of humans; *Capillaria bohmi* in the nasal mucosa of canids; *Trichosomoides crassicauda* in the urinary bladder of rats; *Anatrichosoma* spp. of the skin and nasal mucosa of primates; and *Gongylonema* spp. in the nasal mucosa, oral mucosa, and esophagus of primates and cattle.

AFIP Diagnosis: Skin: Epidermal hyperplasia, diffuse, marked with multifocal degeneration and necrosis, orthokeratotic hyperkeratosis and intraepidermal aphasmid nematodes

Conference Comment: African clawed frogs are found in several areas of Africa south of the Sahara. They are aquatic frogs with several distinct anatomic features including a flattened head and body but no external ear or tongue.[3] Because these frogs have no tongue, they use their unwebbed fingers or front feet to push food into their mouths. Their hind feet are webbed and the inside three toes of each foot have claws from which their name is derived.[3] They can also change color to match that of their environment and they have a lateral line system similar to fish.[3] These frogs are commonly used in biomedical research, so a cursory understanding is important to be able to interpret potential lesions.

Dr. Nichols discussed several aspects of amphibian skin during the conference, and it was noted that the skin of this animal was markedly hyperkeratotic and hyperplastic.

Dr. Nichols opined that frog skin should not be more than a few cell layers thick with sparse to unnoticeable keratin. He also pointed out that frogs perform several physiologic processes with their skin to include respiration, thermoregulation, and electrolyte homeostasis. Due to this complex interaction with the external environment, it is easy to speculate that this type of skin lesion would be enough to cause mortality. Dr. Nichols also referenced the large serous glands containing eosinophilic secretory product in the dermis and the adjacent mucous glands containing clear to slightly amphophilic flocculent material. These glands combine to provide the slime cover of these aquatic amphibians.

Other aphasmids of note in domestic animals that the contributor did not mention include *Trichuris* sp., *Trichinella* sp., *Dioctophyma* sp., and *Eustrongylides* sp.[1]

Contributing Institution: Veterinary Services Center, Department of Comparative Medicine
Stanford School of Medicine, http://med.stanford.edu/compmed/

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CASE III – 080261-01 (AFIP 3113794)

Signalment: Juvenile, undetermined sex, bluegill fish (*Lepomis macrochirus*)

History: A group of juvenile bluegill was purchased from a commercial fish hatchery for use in respiratory physiology studies. The fish were then group-housed in a large indoor water tank. Approximately 2 weeks after their arrival in the laboratory, several fish were noted to have white cutaneous nodules on their fins and/or bodies. Three affected fish were removed from the tank, euthanized, and submitted for necropsy.

Gross Pathology: A 3 mm diameter, firm, white, multilobulated, exophytic, nodular, cutaneous mass protrudes from the dorsal midline of fish #1 at the cranial margin of the dorsal fin **(Fig. 3-1)**. Three smaller (<1mm) white nodules are present along the dorsocaudal aspect of this fish's dorsal fin. Similar nodules of varying sizes and lobulation are located on the caudal peduncle, anal fin, and ventral midline of fish #2 and the tail fin and ventral midline of fish #3.

Laboratory Results: NA

Histopathologic Description: A tranverse section of the whole body is present on the slide. The skin overlying the dorsal midline and, in some slides, including the base of the dorsal fin, contains an irregularly shaped nodular dermal mass measuring up to 2 mm in greatest diameter **(Fig. 3-2)**. This mass contains multiple large round to oval cells **(Fig. 3-3)**, measuring up to 350 µm in diameter, each of which has a 2-10 µm thick lightly basophilic hyaline capsule, abundant amounts of flocculent basophilic cytoplasm, and often a single irregularly shaped degenerate karyomegalic nucleus (up to 80 µm in diameter) containing condensed chromatin and a single large nucleolus. Within the cytoplasm of most of these cells, primarily in a subcapsular location, are irregularly shaped basophilic inclusions that often coalesce to form a fine lattice. Multifocally, there is rupture of the cell capsule and/or intracellular infiltration by macrophages and lymphocytes. The dermis between the large cells is diffusely infiltrated by low to moderate numbers of macrophages and lymphocytes. Multifocally, the overlying epidermis is mildly hyperplastic. However, there are also foci of epidermal erosion.

No significant changes are present in other organs of this fish.

Contributor's Morphologic Diagnosis: Skin; focal (nodular) dermal fibroblast hypertrophy, severe, with karyomegaly, hyaline capsule formation, basophilic intracytoplasmic inclusions, degeneration, lymphohistocytic inflammation, epidermal hyperplasia, and epidermal erosion

Contributor's Comment: All histology slides submitted for this case are from fish #1, and the lesion present is depicted grossly in Figure 3-1. The gross and histologic findings are consistent with the fish disease known as "lymphocystis."[5] The severely hypertrophied dermal fibroblasts with hyaline capsules and basophilic intracytoplasmic inclusions are pathognomonic for this disease and are known as "lymphocystis cells"; an individual cell, which may enlarge up to a million (10^6)

3-1. Bluegill. A firm, white, multilobulated exophytic cutaneous mass protrudes from the midline at the cranial aspect of the dorsal fin. Image courtesy of US Army Medical Research Institute of Infectious Diseases, ATTN: MCMR-UIP, 1425 Porter Street, Fort Detrick, MD 21702, donald.k.nichols@amedd.army.mil.

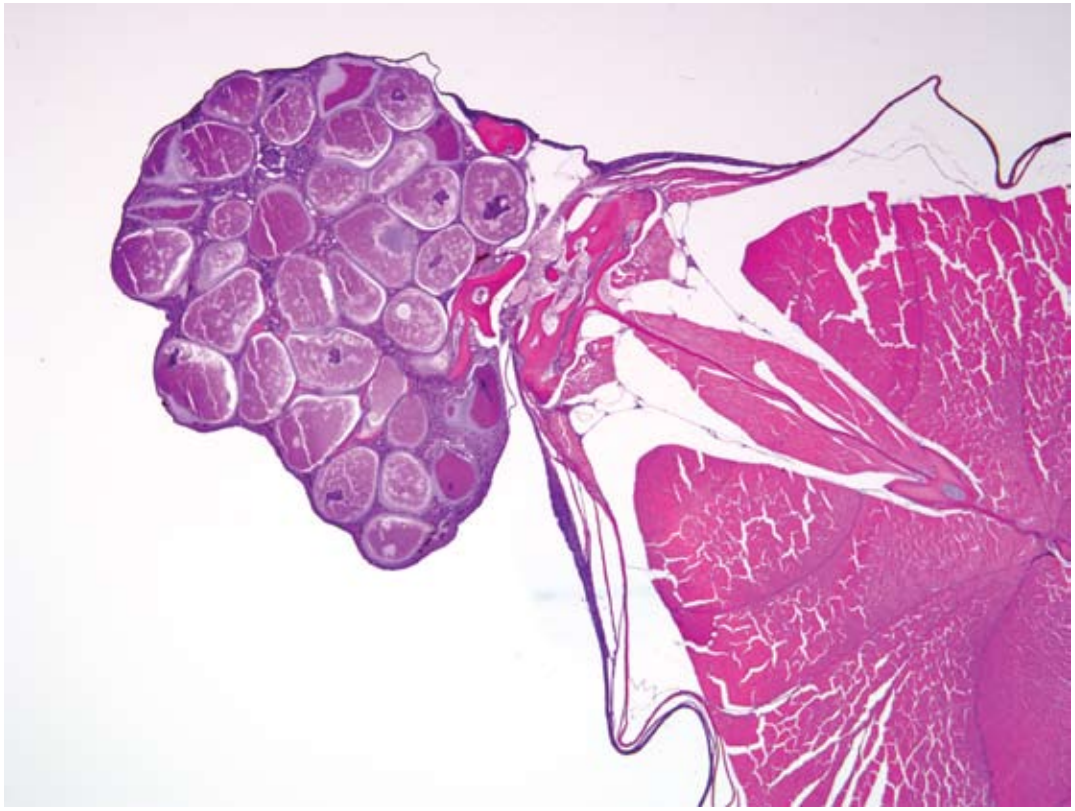
times normal size, appears grossly as a cream-colored nodule or "lymphocyst" measuring up to 2 mm in diameter.[1] Nodules may occur singly or grouped together in raspberry-like clusters (as in this lesion). These nodules are most commonly seen on the fins and body. Occasionally, the gills, peritoneum, and/or internal organs may be affected.[1,5]

Lymphocystis is a common and widespread disease of teleost fish that has been reported in more than 140 species of freshwater and marine fish.[1] Species in the order Perciformes (which includes bluegill) and order Pleuronectiformes ("flatfish" such as flounder, sole, plaice, and dab) constitute approximately 75% and 10%, respectively, of the species affected.[1]

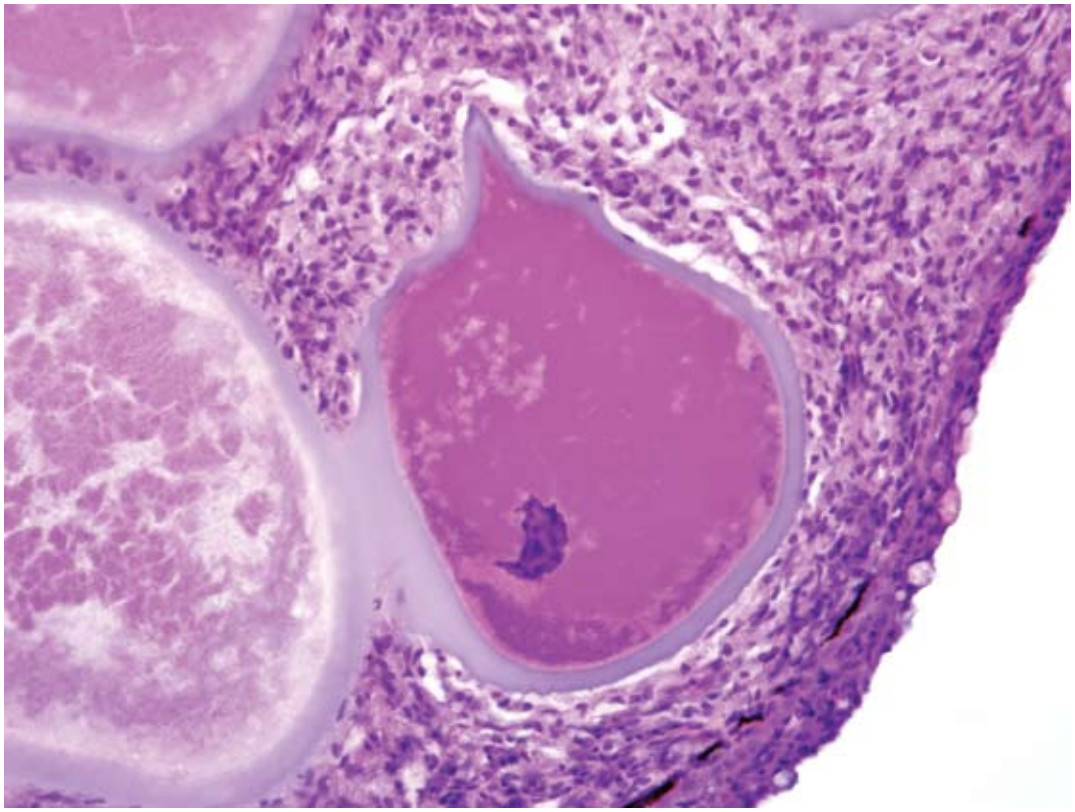
This disease is caused by infection with lymphocystis disease virus (LDV), which is a DNA virus in the genus *Lymphocystivirus* of the family Iridoviridae.[3] Currently, there are two recognized species of LDV: LDV-1 and LDV-2.[3] However, there appear to be different strains of the viruses that vary in size and host specificity.[1,5] Viral particles are non-enveloped and display icosahedral symmetry; by transmission electron microscopy (TEM), they usually appear hexagonal and may form paracrystalline arrays within the cytoplasm of infected cells – as seen in one of the lesions removed from fish #3 **(Fig. 3-4)**.

Viral infection may occur through the gills, digestive tract, or skin wounds.[1] Infected fibroblasts soon begin to enlarge; increases in cytoplasm are accompanied by nuclear and nucleolar enlargement.[5] As the cell enlarges, irregularly shaped basophilic cytoplasmic inclusions develop; TEM reveals that these are sites of viral assembly.[1] The characteristic hyaline capsule of a lymphocystis cell appears at approximately the mid-stage of maturation.[5] Cellular degeneration begins with nuclear condensation followed by breakdown of the capsule; infiltrates of macrophages and lymphocytes surround and eventually invade degenerate cells.[5] Rupture of the degenerate cells releases the viral particles which may then infect adjacent fibroblasts or, if released into the environment, infect other fish.[1,5]

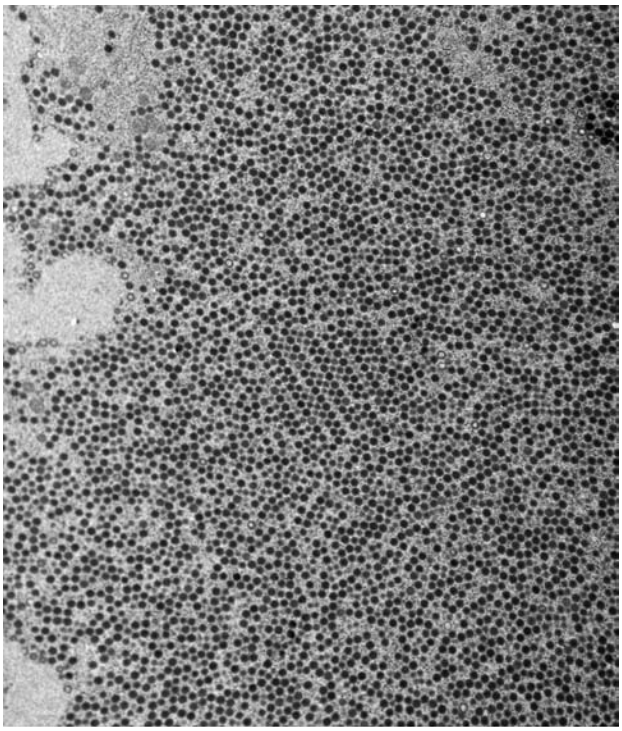
Lymphocystis is a benign disease with very low mortality; lesions usually heal completely. However, secondary bacterial or fungal infection of the lesions may result in debilitation and/or death.[1]



3-2. Skin, bluegill. The skin overlying the dorsal midline is markedly expanded by a nodular dermal mass measuring 3 mm in diameter which is composed of multiple large round to oval cells. (HE 20X)



3-3. Skin, bluegill. Dermal mass is composed of multiple hypertrophied fibroblasts measuring up to 350 um in diameter which have a 2-3 um hyaline capsule, abundant amounts of flocculent amphophilic cytoplasm, irregular basophilic cytoplasmic viral inclusion material, and an irregularly shaped karyomegalic nucleus. The remaining dermis between the hypertrophied cells contains moderate numbers of macrophages and lymphocytes. (HE 400X)



3-4. Skin, bluegill. The cytoplasm of a fibroblast contains numerous hexagonal viral particles which are arranged in paracrystalline arrays. Electron micrograph courtesy of US Army Medical Research Institute of Infectious Diseases, ATTN: MCMR-UIP, 1425 Porter Street, Fort Detrick, MD 21702 donald.k.nichols@amedd.army.mil.

Interestingly, the first isolation and in vitro cultivation of LDV was done with samples collected from infected bluegill and the first experimental pathogenesis studies were performed with this fish species.[2] In these studies it was found that for fish kept at 25ºC the time from experimental infection to lesion regression was approximately 28 days.[2] In a later study with experimentally infected plaice kept at 10ºC, it took 3 months for the lesions to regress.[5]

After the diagnosis of lymphocystis in the three bluegill described above, the following measures were taken to control the disease in the remaining group of fish: 1) the fish were closely examined and each one with visible lymphocystis lesions was removed and euthanized; 2) water flow through the holding tank was increased to flush out any viral particles in the tank; and 3) the temperature of the water in the tank was increased to 25ºC to enhance the immune function of the fish and inhibit virus "survival" in the tank environment. These control measures were successful; no additional cases of lymphocystis have since been identified in these fish.

AFIP Diagnosis: Scaled skin: Fibroblast hypertrophy, nodular, focal with karyomegaly, basophilic cytoplasmic inclusions and moderate lymphoplasmacytic dermatitis

Conference Comment: The contributor did an outstanding job of covering all the salient features of lymphocystis. Viruses in the family *Iridoviridae* generally affect fish, amphibians and invertebrates. Within the family there are 5 genera: 1) *Iridovirus* (the iridoviruses); 2) *Chloriridovirus* (large iridescent insect viruses); 3) *Ranavirus* (frog iridoviruses); 4) *Lymphocystivirus* (lymphocystis viruses of fish); and 5) unnamed (goldfish iridoviruses). African swine fever was once considered part of the *Iridoviridae* family, but it has recently been recategorized into its own family.

Contributing Institution: US Army Medical Research Institute of Infectious Diseases, Pathology Division, 1425 Porter Street, Fort Detrick, MD , http://www.usamriid.army.mil/

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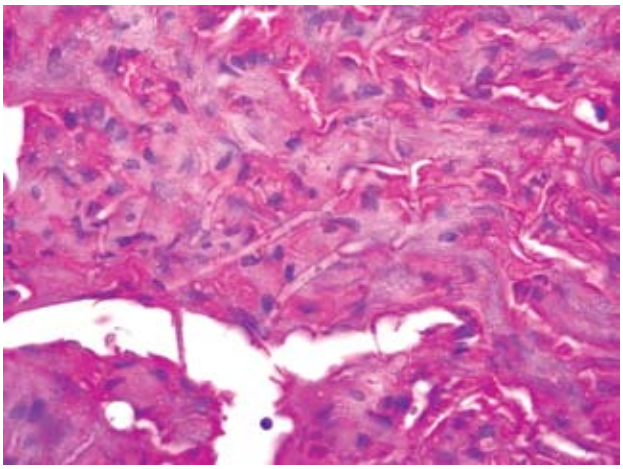
CASE IV – 48928 (AFIP 3026807)

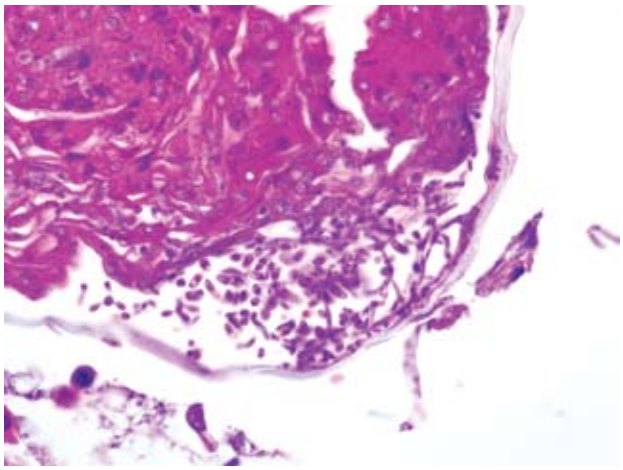
Signalment: Five-year-old, captive hatched, male tentacled snake (*Erpeton tentaculatum*)

History: One of a group of tentacled snakes with a 3 or 4 month history of skin lesions. Six snakes died despite treatment.

Gross Pathology: Numerous 2-4 mm diameter irregular slightly depressed rough tan lesions are scattered in the



4-1. Skin, tentacled snake. Multifocally, the epidermis is replaced by a necrotic coagulum which contains numerous branching septate fungal hyphae. (HE 1000X)



4-2. Skin, tentacled snake. Rarely admixed with the fungal hyphae are numerous arthroconidia. (HE 1000X)

skin of the entire body.

Laboratory Results: Fungal culture of skin grew *Chrysosprorium* anamorph of *Nannizziopsis vriesii*

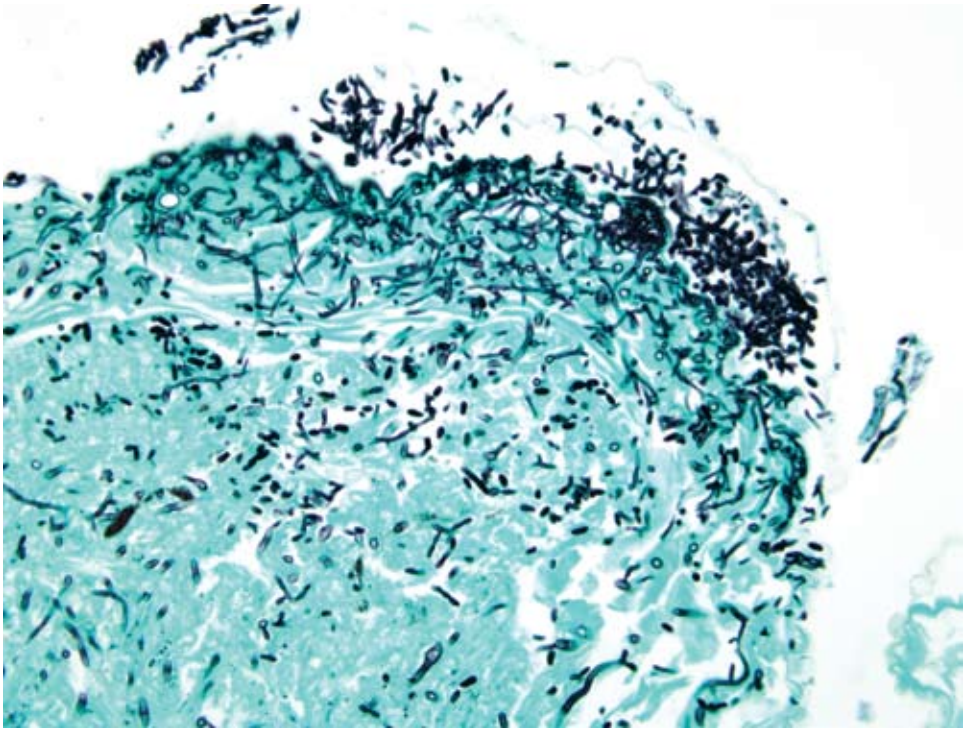
Histopathologic Description: Multifocally, there is full-thickness epidermal necrosis with invasion and expansion of the necrotic tissue by numerous branching, septate fungal hyphae **(Fig. 4-1)** and rare arthroconidia **(Fig. 4-2)**. In some affected areas, myriads of mixed bacteria are also present. Heterophils multifocally infiltrate the epidermis and dermis and are especially abundant in the dermis in areas where there is separation or loss (ulceration) of the necrotic epithelium. Less affected epidermis is diffusely mildly hyperplastic. Spongiosis and cytoplasmic vacuolation are common.

Contributor's Morphologic Diagnosis: Skin: Severe multifocal ulcerative and necrotizing dermatitis with intralesional fungi (etiology: *Chrysosprorium* anamorph of *Nannizziopsis vriesii*)

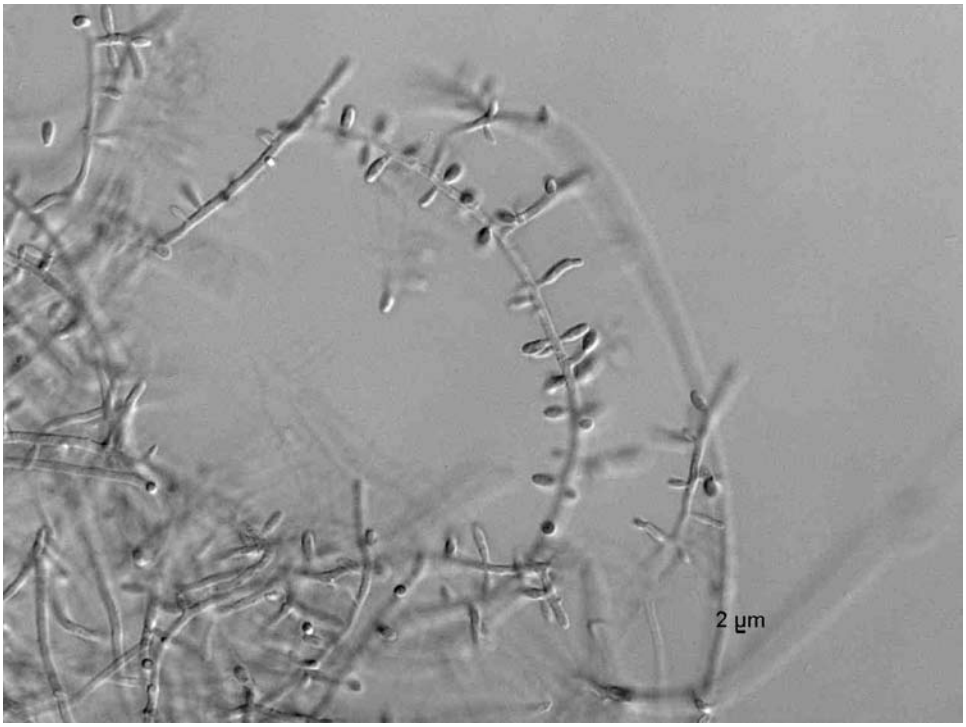
Contributor's Comment: The *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) has relatively recently been recognized as a pathogenic fungus that can cause significant morbidity and mortality in some species of reptiles.[2,4,6] In previous reports, dermatomycoses in snakes and lizards have been ascribed to several different species of fungi including dermatophytes such as *Geotrichium* sp., *Trichosporon* sp., and numerous other soil fungi (e.g., *Aspergillus, Candida, Cladosporium, Fusarium*, and *Mucor*).[2,4,6] It is proposed that in many of these cases the fungus was misidentified and infection was due to the CANV.[2,4] *Nannizziopsis vriesii* is a sexually reproductive keratinophilic species in the phylum Ascomycota, order Onygenales, that was originally isolated from the tissues of a lizard (*Ameiva* sp.).[4,6] Sexual fruiting bodies are formed on nutrient-poor medium at 30 degrees C. The asexual (mitotic) state seen in histologic lesions is typical of *Chrysosporium* and produces solitary conidia (aleurioconidia) and arthroconidia. Although culture is necessary to definitively identify the CANV from lesions in affected reptiles, the characteristic appearance of the arthroconidia in histologic section is a key diagnostic feature. The CANV was cultured from the skin of the snake submitted for this conference (Fig. 1). Arthroconidia were not seen histologically in this snake but were seen in the skin lesions of other snakes in this group (Fig. 2). The origin of infection with the CANV in this and other cases is unknown. An extensive survey of skin samples from healthy reptiles did not recover any of the CANV.[7] Thus, *N. vriesii* does not appear to be a common constituent of the microflora of healthy reptiles. An environmental source is postulated but has not been demonstrated. Factors that might predispose a reptile to infection and disease are also not known.

AFIP Diagnosis: Scaled skin: Epidermitis, necrotizing and ulcerative, multifocal, marked with intralesional fungi

Conference Comment: Within the last 10 years, CANV has been identified as the causative agent of dermatomycosis in several reptilian species to include green iguanas, bearded dragons, brown tree snakes, a salt-water crocodile, and veiled chameleons as well as



4-3. Skin, tentacled snake. GMS positive branching fungal hyphae within the necrotic epidermis. (GMS 400X)



4-4. Skin, tentacled snake. Fungal arthroconidia isolated from culture. Photograph courtesy of Zoolocial Society of San Diego, P.O. Box 120551, San Diego, CA 92112-055, pathology@ sandiegozoo.org.

tentacled snakes.[1,2,3,4,5,8] A necrotizing and granulomatous dermatitis is commonly seen in these species with some species variation.[1,2,3,4,5,8] One of the bearded dragons infected with CANV also had a granulomatous hepatitis with intralesional hyphae.[3] Histologically, CANV is found in necrotic lesions and appears as hyaline, septate, branching hyphae often 2-4 um in width with characteristic arthroconidia.[2]

In the case of the tentacled snakes, it was speculated by

the author of the article that failure to maintain an acidic environment predisposed these snakes to skin infection. These snakes normally inhabit slow moving acidic streams at a pH of 6-6.5, and the affected snakes were kept in water at a pH of > 8.(2) Snakes kept in water at a pH of 7 did not develop lesions.[2]

The morphologic features of CANV in tissue section are best demonstrated with special stains such as GMS **(Fig. 4-3)**. The contributor submitted a superb image of hyphae and arthroconidia growing in culture **(Fig. 4-4)**.

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