Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 10

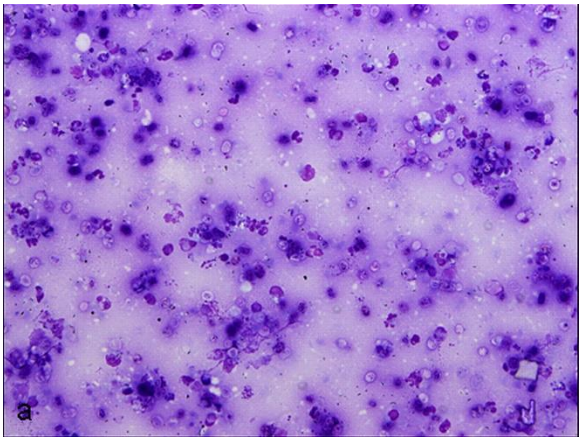
28 November 2018

CASE I: Case 1 (JPC 4006380)

Signalment: 3-year old, boxer, dog (*Canis familiaris*)

History: The patient initially presented to the internal medicine service at the University of Glasgow with a 1 year history of protracted colitis. An endoscopic biopsy of the colon was taken at that time. Five weeks following biopsy, the patient re-presented for acute onset of blindness. The owners reported a slight vision loss beginning roughly 1 month prior that progressed rapidly to complete blindness within the previous 24 hours.

On physical examination, there was subtle ocular hyperemia. The menace responses were absent and the PLRs very weak and sluggish. There was mild corneal edema and significant deep stromal vascularization in both eyes. There was marked aqueous flare and swollen irides. The right eye had a reddish tapetal reflex with a blood tinged



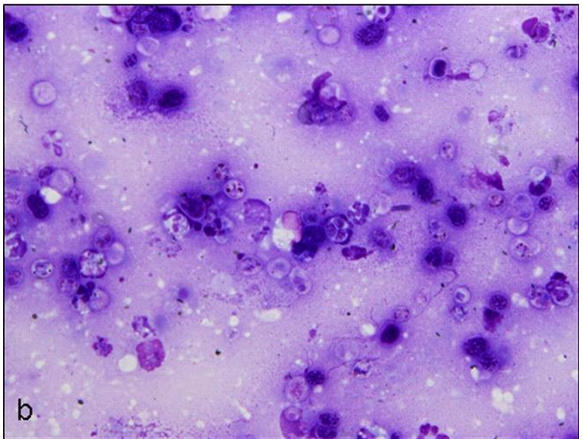
Subretinal aspirate, dog: A good quality, moderately cellular aspirate on a proteinaceous background is submitted. At this magnification, degenerate neutrophils are evident and a single rhomboidal cholesterol crystal is present at bottom left. . (Photo courtesy of: School of Veterinary Medicine, University of Glasgow, Garscube Estate, Bearsden, Glasgow, G611QH, United Kingdom, http://www.gla.ac.uk/schools/vet/) (May-Grunwalds, 100X)

and murky posterior segment with no detail discernible. The left eye had a good tapetal reflex but there was profound posterior segment disturbance with thickened folds of hyperemic and hypervascular detached retina present throughout and the impression of a cellular retinal infiltrate. A subretinal aspirate was collected and submitted to Veterinary Diagnostic Services at the University of Glasgow for cytologic analysis and routine cultures. Cytology smears were routinely stained with May-Grünwalds and Giemsa.

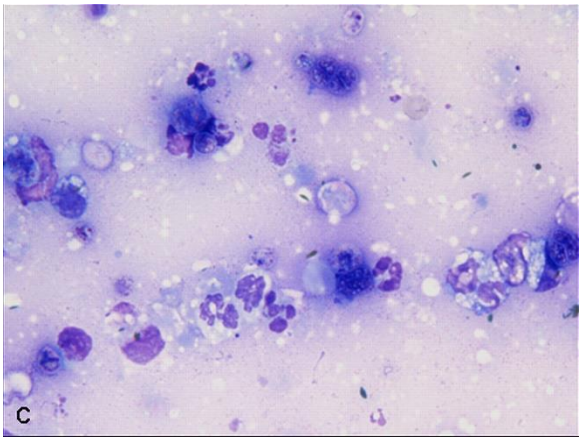
Gross Pathology: None given.

Laboratory results: *Prototheca* sp. was cultured on routine aerobic cultures. The organism was not further speciated.

Cytologic Description:
Examined is a highly cellular smear of good quality. There is a dense, faintly basophilic proteinaceous background containing many free rod-shaped melanin granules. There are large numbers of degenerate neutrophils admixed with low numbers macrophages and occasional erythrocytes. Abundant intracellular and extracellular algal organisms are present, both intact and as



Subretinal aspirate, dog: Individual alga (sporangia) and clusters of deeply basophilic proliferating endospores are present among the inflammatory cells. A single sporangiospore at center exhibits tripartite division characteristic of Prototheca (arrow). (Photo courtesy of: School of Veterinary Medicine, University of Glasgow, Garscube Estate, Bearsden, Glasgow, G611QH, United Kingdom, http://www.gla.ac.uk/schools/vet/) (May-Grunwalds, 200X)



ruptured empty cell casings. Algal

Subretinal aspirate, dog: Neutrophils contain vacuolated cytoplasm and degeneration is characterized by hypersegmented, hyperchromatic and mildly shrunken nuclei. At left, a macrophage contains an engulfed alga. Empty cyst forms are scattered throughout the image. Rod-shaped melanin granules are present within the proteinaceous background. (Photo courtesy of: School of Veterinary Medicine, University of Glasgow, Garscube Estate, Bearsden, Glasgow, G611QH, United Kingdom, http://www.gla.ac.uk/schools/vet/) (May-Grunwalds, 400X)

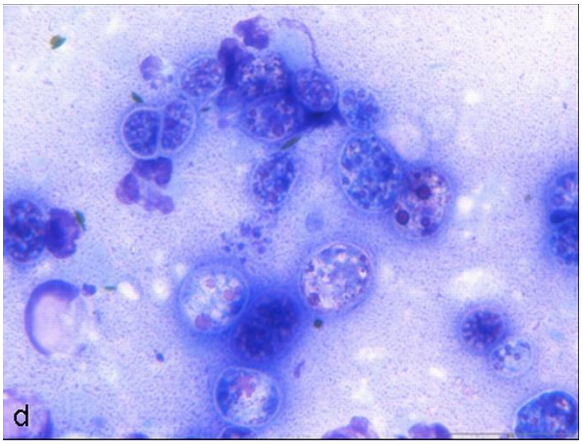
organisms are variably sized, 5-10 µm diameter, round to oval, have a thin, clear cell wall and have deeply basophilic cytoplasm (consistent with *Prototheca* spp.). Early endospore replication is characterized by clusters of 3-5 endospores with deeply magenta nuclei and internal septation. Sporangia are 20 µm in diameter, have pale basophilic cytoplasm and contain up to 20, magenta, 5 µm diameter sporangiospores. Occasional organisms are seen intracellularly within the cytoplasm of degenerate cells. Organisms are Periodic Acid-Schiff (PAS) positive.

Contributor's Morphologic Diagnoses: Purulent inflammation with extracellular algal organisms (consistent with *Prototheca* spp.)

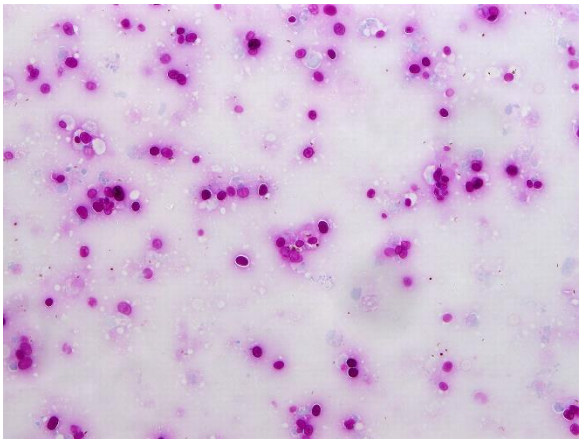
Contributor's Comment: *Prototheca* spp. are unicellular, colorless, saprophytic algae

that can be found within the environment, particularly with standing water and sewage effluent.[6] Protothecosis is a rare disease of people and animals that can be localized, such as in the intestine or skin, or disseminated, resulting in widespread granulomatous and/or necrotizing lesions throughout the body.[1] In people, infection is primarily within the skin or joints. [6] Protothecosis is a relatively uncommon cause of mastitis in cattle.[1] In dogs, the intestine and the eye are the most common organs affected. [2,5,6]

Colitis is the most common presenting complaint in dogs with protothecosis and the clinical course is often protracted. [6] Dogs can also present with acute blindness.[9] The dog in this case initially presented with colitis, followed by acute blindness and had a clinical presentation typical of protothecosis. In dogs, protothecal organisms first invade and colonize the colon, with further spread through both lymphatics and blood vessels to other organ systems. In this case, biopsies from the colon in this dog revealed a marked pyogranulomatous and ulcerative colitis.



Subretinal aspirate, dog: Clusters of deeply basophilic endospores and interspersed with larger individualized sporangia containing 2-3um eosinophilic sporangiospores. (Photo courtesy of: School of Veterinary Medicine, University of Glasgow, Garscube Estate, Bearsden, Glasgow, G611QH, United Kingdom, http://www.gla.ac.uk/schools/vet/) (May-Grunwalds, 600X)



Subretinal aspirate, dog: Algae stain positively with a periodic acid-Schiff preparation. (Photo courtesy of: School of Veterinary Medicine, University of Glasgow, Garscube Estate, Bearsden, Glasgow, G611QH, United Kingdom, http://www.gla.ac.uk/schools/vet/) (PAS, 100X)

Rare protothecal organisms were detected within the sections, confirming infection in both the intestine and the eye. The dog initially responded to treatment with partial resolution of the colitis, which included amphotericin B and itraconazole; however, vision never returned. The dog was eventually euthanized due to financial constraints. Disseminated infection could not be documented because post-mortem examination was declined.

Protothecal organisms can be highlighted with special stains, such as PAS and Gomori's Methenamine-Silver, in both cytological and histopathology sections. [1] Algal organisms of the *Chlorella* species can be morphologically similar to *Prototheca* spp. *Chlorella* spp. can be differentiated by the often green tinge to the organisms and the presence of PAS positive cytoplasmic granules that are negative following amylase digestion.[1] The two main species of *Prototheca* known to cause infection in dogs are *P. zopfii* and *P. wickerhamii*. Though these species of *Prototheca* differ slightly morphologically (size and number of sporangiospores), PCR is required for definitive differentiation.

Differentials for suppurative or pyogranulomatous inflammation in ocular fluid include both bacterial and fungal infections. [9] Rarely, protozoa, such as *Toxoplasma gondii*, can result in endophthalmitis.[9] Any case of bacteremia has the potential to develop bacterial endophthalmitis and the list of potential agents is extremely long.[9] Fungal endophthalmitis is also typically secondary to a mycotic septicemia. Fungal organisms most commonly associated with eye infections include *Cryptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum* (particularly in cats), and, less likely, *Coccidioides immitis*.[9] Both bacterial and fungal infections of the eye are often differentiated based on morphology of the organism, including size and presence of budding, and/or culture. Cultures can be obtained from endoscopy samples, ocular fluid, and/or urine of living patients, depending on the organ systems involved clinically.

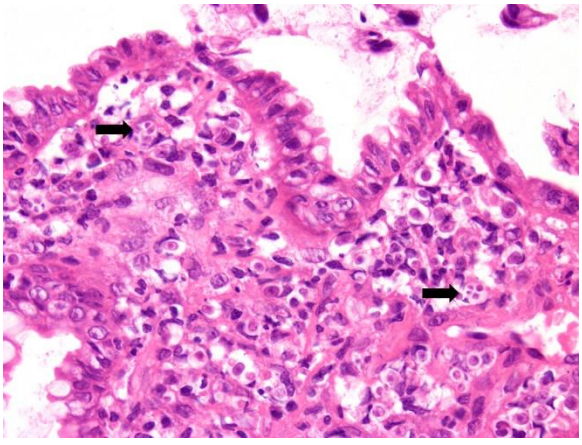
Contributing Institution:
School of Veterinary Medicine
University of Glasgow
Garscube Estate
Bearsden, Glasgow
G611QH, United Kingdom
http://www.gla.ac.uk/schools/vet/

JPC Diagnosis: Fine needle subretinal aspirate: Pyogranulomatous inflammation with numerous endosporulating alga.

JPC Comment:
Four species of *Prototheca* exist: *P. zopfii*, *P. wickerhamii* (the two pathogenic forms), *P. stagnora*, and a proposed form, *P. salmonis*. They are found in abundance in nature in animal waste, sewage, soil, food, and flowing or standing water and have a worldwide distribution. Additional ribosomal sequencing further divided *P. zopfii* into biotypes, with *P. zopfii* biotype one being present in bovine liquid manure, biotype 2 present in many cases of bovine mastitis, and biotype 3 isolated from swine manure (and recently renamed *P. blaschkeae.)*[4] A novel *Prototheca (P. cutis* sp. nov) has been tentatively identified based on ribosomal RNA sequencing of a single human isolate.[4]

Despite its ubiquity, animal infections are rare, with dogs, cats and cattle predominantly reported in the literature. Cats are most commonly infected cutaneously with large firm nodules on the limbs and feet, and *P. wickerhamii* is most commonly isolated.[7] In cattle, *P. zopfii* is an uncommon cause of pyogranulomatous lesions of the bovine mastitis and associated lymph nodes.[4] In goats, *P. wickerhamii* causes ulcerative nodules of the mucocutaneous junction of the nasal vestibule, nostrils, and subcutis of the face and head.[4]

Human infections are uncommon and usually manifest as erythematous vesicobullous rashes which may occasionally be crusting and purulent. Risk factors include immune suppression from HIV, prolonged steroid use, diabetes



Colon, dog: Numerous algal life stages (arrows) are admixed with neutrophils, macrophages, lymphocytes, and plasma cells (HE, 400X). (Photo courtesy of: School of Veterinary Medicine, University of Glasgow, Garscube Estate, Bearsden, Glasgow, G611QH, United Kingdom, http://www.gla.ac.uk/schools/vet/) (PAS, 100X)

mellitus, and underlying malignancy.[2] The frequency of cases increases with age. Most human cases are the result of infection with *Prototheca wickerhamii*. Disseminated infections, although uncommon and usually seen only in patients with severe immunodeficiency, and demonstrate a mortality rate of 60% (in a limited number of reported cases.[2]

A related non-photosynthetic of algae, *Helicosporidium*, has recently been identified as a specialized parasite from a variety of dipteran, coleopteran, and lepidopteran insects, primarily infecting larvae.[8]

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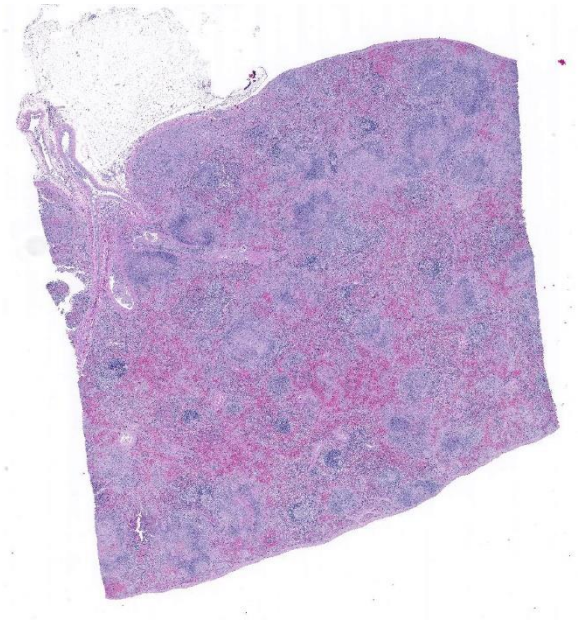
CASE II: 9428-12 (JPC 4048660).

Signalment: Two-years-old, neutered, male, domestic shorthair cat, (*Felis domesticus)*

History: Patient presented to emergency clinic in lateral recumbency with oral ulcers, mild dyspnea, hypothermia, and mild nystagmus. Reportedly, the patient had been anorexic. The patient died within one hour of presentation.

Gross Pathology: Spleen was enlarged with a roughened serosal surface. Multiple white pinpoint foci were observed on the serosal surface. The cut surface bulged and had a granular appearance. Liver was swollen with rounded margins and multiple white pinpoint foci were present on the serosal and cut surfaces.



Spleen, cat. At subgross, pallor (necrosis) of the white pulp, extending into the adjacent red pulp is prominent.

Laboratory results: Neutropenia, hyper-bilirubinemia, elevated ALT. *Francisella tularensis* was isolated from the spleen and from a lymph node sample.

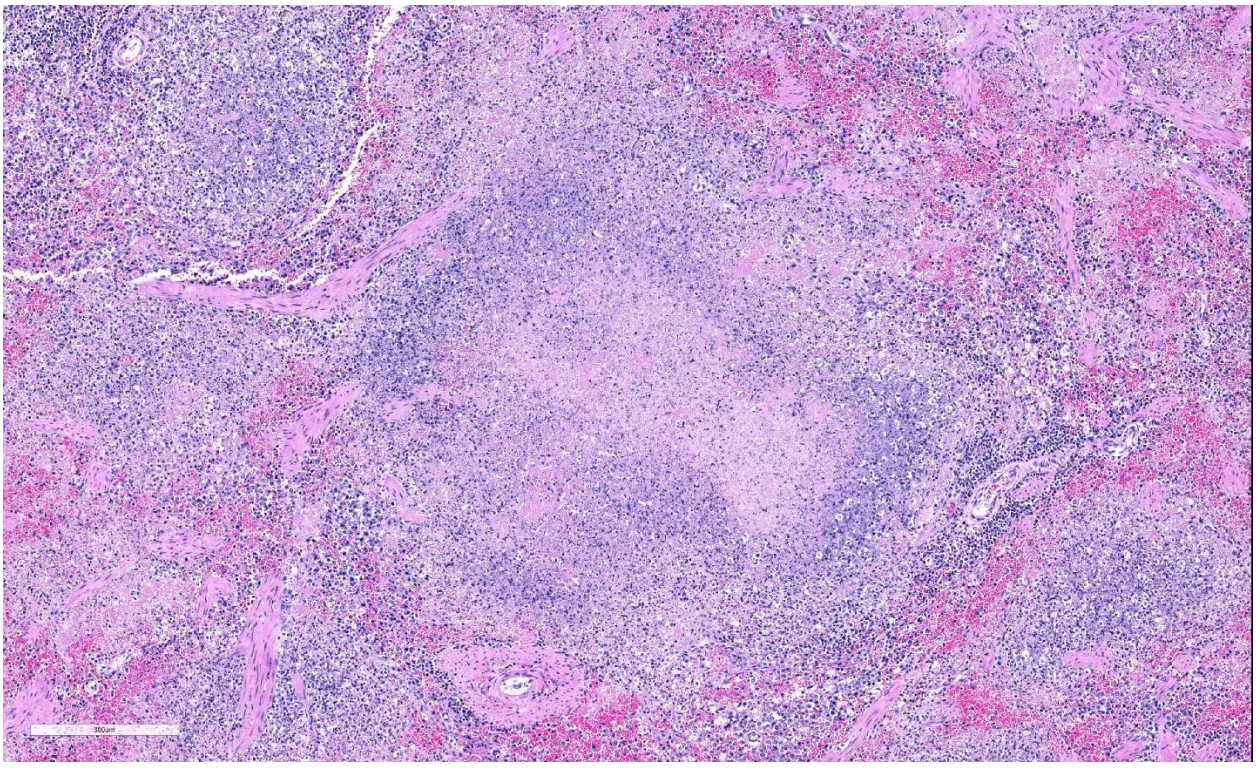
Microscopic Description:
In sections of spleen, there is disseminated and coalescing necrosis of germinal centers which extends into adjacent red pulp. Foci of necrosis are accompanied by an inflammatory response comprised of neutrophils and macrophages. Scattered thrombosed blood vessels can be seen mainly associated with the capsule and near the hilus. Variably sized and shaped aggregates of homogenous eosinophilic material is present in many germinal centers. Mesothelial cells on the serosa are reactive characterized by cuboidal appearance.

Contributor's Morphologic Diagnoses:
1. Severe, subacute, multifocal, coalescing, necrotic, splenitis.
2. Splenic amyloidosis – germinal centers

Contributor's Comment: *Francisella tularensis* is a gram-negative, encapsulated, facultative intracellular pathogen.[7] *F. tularensis* is subdivided into two subtypes. Type A is *F. tularensis* subsp. *tularensis* and has and infectious dose in humans of <10 CFU's, whereas type B is *F. tularensis* subsp. *holarctica* which as an infectious dose of $<10^3$ CFU and a milder form of tularemia in humans.[7] The organism is abundant in nature and infects many mammalian and arthropod species.[11] *F. tularensis* type A has been isolated from cats on numerous occasions and can be transmitted from cats and other animals (deer, personal experience) to humans. [5,10,13]

Diagnosis, in some cases may be difficult, but culture appears to be more sensitive than immunohistochemistry.[1] Gross lesions consist of multiple pinpoint white foci on the spleen, liver, and lymph nodes. As a facultative intracellular parasite, it may persist for years as a latent infection.[11] The genes for several virulence factors have been identified and shown to share some features with the intracellular parasite, *Listeria monocytogenes*.[1] Tularemia in other mammalian species such as horses and sheep are often associated with heavy infestation by ticks such as *Dermacenter andersoni* and *Amblyoma americanum*.[12] One serologic survey indicated 12 – 24 percent of cats had antibodies to *F. tularensis* due to natural exposure.[6] Those serologically positive animals were negative for *F. tularensis* DNA, indicating infection may be may have been cleared naturally. Tularemia should be considered in a differential diagnosis of unexplained febrile illness in cats.

Amyloidosis in the spleen is considered an incidental finding in this case. Amyloidosis of splenic germinal centers is reportedly a



Spleen cat. At higher magnification, white pulp is largely effaced by lytic necrosis extending into the surrounding red pulp. There is abundant fibrin in the adjacent necrotic red pulp, replacing (HE, 93X)

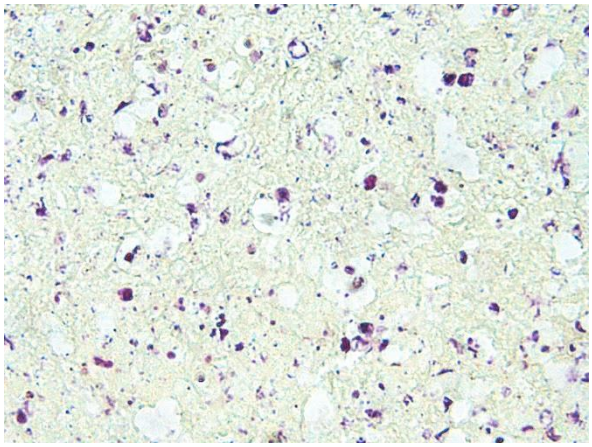
rare finding in cats and particularly domestic shorthair cats.[11] Generalized amyloidosis is more common in Abyssinian cats and has been reported in Siamese cats and wild black-footed cats as well.[9]

Contributing Institution:
Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, NE. vbms.unl.edu/nvdls

JPC Diagnosis: Spleen, lymphoid tissue: Necrosis, diffuse, with multifocal red pulp necrosis, hemorrhage, fibrin, and edema.

JPC Comment: *Francisella tularensis* is a global pathogen that has been classically divided into three distinct subspecies: *F. tularensis* subsp. *tularensis*, *F. tularensis* subsp. *holarctica*, and *F. tularensis* subsp. *mediasiatica.* A fourth, *F. tularensis* subsp. *novicida*, which is non-pathogenic for humans, was recently separated into a separate species, *F. novocida*, and is extensively used in *Francisella* research.[7] Additional research has divided *F. tularensis* into genetically distinct clades, Type A and Type B, with the highly pathogenic type A (*F. tularensis* subsp. *tularensis)* found in the USA and the less virulent type B (*F. tularensis* subsp. *holarctica)* found throughout the Northern Hemisphere.[7] The first report of *F. tularensis* subsp. *holarctica* in Australia was recently published.[3] Both Type A and Type B infections of *F. tularensis* subsp. *tularensis* have been identified in cats.[12]

The first case of *Francisella tularensis*-mediated disease was recognized as a plague-like illness in rodents during an outbreak in Tulare County, California in 1911, and the first human cases were described three years later in two patients in Ohio, who had recently had contact with wild rabbits.[4] In 1919, Dr. Edward Francis



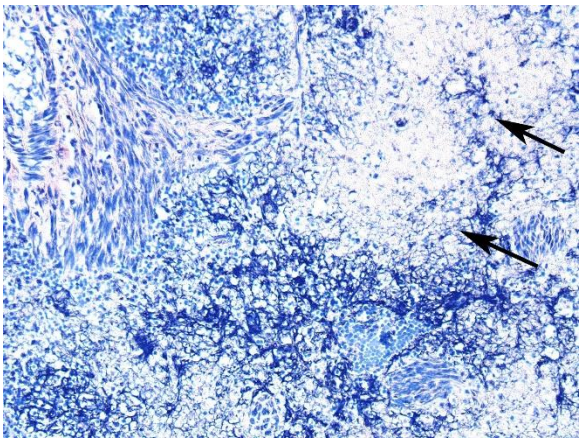
Spleen, cat. Areas of necrosis contain large numbers of small 1-2um gram-negative coccobacilli. (Brown-Hopps, 600X).

established that the disease was caused by *Bacterium tularensis* (named for the county in which the disease was originally identified) and appended the name "tularemia" to the disease.[4] The number of cases of tularemia in the US peaked in 1939-1940 with 2,291 documented cases, but has decreased to a current average of 125 cases over the last two decades.[8] Tularemia cases in both humans and animals have been identified in 49 US states; with Hawaii being the only exception.[8]

While the transmission of tularemia has classically been associated with contact with rodents and rabbits, giving rise to the colloquialisms "rabbit fever", "market men's disease", and "meat-cutter's disease"[7], a number of important methods of transmission also exist: transmission by insect vectors including ticks, biting flies, and mosquitoes, and water-borne outbreaks.[7] Aerosol-born outbreaks have accounted for some of the largest number of cases, from a 1967 outbreak and a forming area of Sweden involving more than 600 individuals[7], as well as a more recent outbreak in Martha's Vineyard, which involved 15 individuals and 1 fatality, with a common factor of lawn mowing or brush cutting a week prior to illness[4].

There are three basic clinical syndromes associated with tularemia: pneumonic (exemplified by aerosol born disease as previously described), typhoidal, and ulceroglandular. Typhoidal tularemia is the result of entry of the bacteria into breaks in the skin or mucous membranes, with vascular dissemination, sepsis, and necrosis in any involved organs (as exemplified in this case). Approximately three quarters of tularemia cases in the US are localized, manifested by a tender regional lymph node a local papule which involves to a chancriform lesion; such cases are referred to as ulceroglandular tularemia.[12] A single case of localized cutaneous infection resembling ulcero-glandular disease has been reported in a cat with localized swellings of the right submandibular salivary gland and mandibular lymph node but lack signs of systemic illness.[10]

Classic association with rodents and rabbits is woefully inadequate to describe the potential for transmission from infected mammalian species. Bites, especially from asymptomatic individuals other than cats may transmit the disease, and human cases have been documented from the bites of



Spleen cat. Areas of necrosis (arrows) are surrounded by abundant polymerized fibrin and hemorrhage. (PTAH, 100X)

dogs, coyotes, raccoon, skunks, opossums, squirrel monkeys, tamarins, and swine.[2]

F. tularensis has an interesting method of surviving macrophage ingestion. Following internalization into phagocytic cells, the bacilli reside in membrane-bound endosomal compartment known as the *F. tularensis*-containing vacuole (FCV) which acquires both early-and late-lysosomal markers but not acid hydrolase cathepsin-D, preventing its fusion with lysosomes, and escaping the bactericidal respiratory burst.[7]

Within the laboratory, *F. tularensis* requires the use of a minimum standard biosafety level II has recommended by the American Society for microbiology. Biosafety level III facility some protocols are recommended for handling. All suspect cultures are isolated, experimental animal studies, and animal autopsies.[8]

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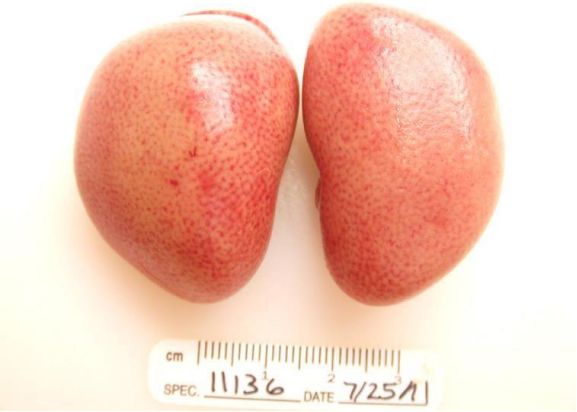
CASE III: 11136 (JPC 4066393).

Signalment: 21-year-old male Indonesian origin cynomolgus macaque (*Macaca fascicularis*).

History: Following sedation for an experimental procedure two weeks prior to death, this female macaque lost 6% of its body weight. It had a 4 year history of eating an atherogenic diet and was in obese body condition. The day prior to death, the animal presented with lethargy, dehydration and hypothermia (94.4˚F). Following an unsuccessful attempt at supportive care, the animal died and was submitted for necropsy.



Kidney, cynomolgus macaque. The renal cortices are diffusely pale tan. (Photo courtesy of: Wake Forest School of Medicine, Department of Pathology/Comparative Medicine, Medical Center Boulevard, Winston Salem, NC 27157-1040 http://www.wakehealth.edu/School/Comparative-Medicine/Training-Programs/ACVP.htm

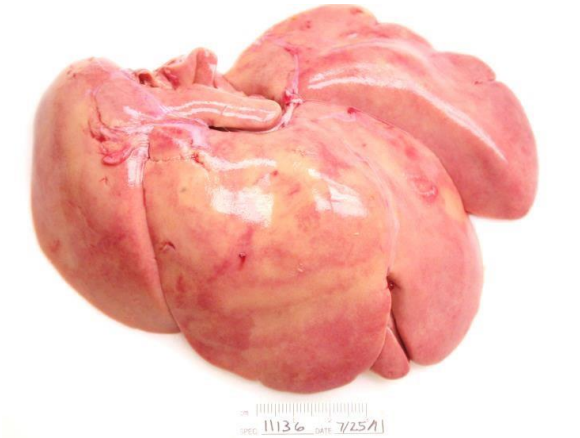
Gross Pathology: The animal was obese with abundant visceral and subcutaneous adipose tissue. The kidneys were diffusely tan. The liver was diffusely pale yellow with a slight reticular pattern, had slightly rounded edges, and cut sections floated in formalin. The luminal surface of the aortic intima was irregularly thickened by pale yellow to tan plaques (atherosclerosis). The stomach and small intestine contained only clear mucinous fluid, flecked with ingesta.

Laboratory results: Blood chemistry values acquired the day prior to death revealed marked azotemia (BUN 107mg/dL; creatinine 8.4mg/dL) with hyperphosphatemia (13.60mg/dL), mildly elevated ALP (239U/L), and moderate hypoproteinemia (total protein 5g/dL; albumen 2.1g/dL).

Microscopic Description:
The renal cortical proximal tubular epithelial cells were markedly dilated by variably-sized clear cytoplasmic vacuoles (lipid), up to 40 microns in diameter. Tubular lumina were often reduced by these plump cells and occasionally contained neutrophils or sloughed epithelial cells. The epithelial cells in some tubules were attenuated and widely spaced indicative of epithelial loss. Scattered tubules contained round lamellar basophilic material (mineral). A few glomeruli had thickened basement membranes and, rarely, glomeruli were atrophic.

Contributor's Morphologic Diagnoses:
Kidney: Vacuolar degeneration, diffuse, marked with epithelial loss and intraluminal mineral, renal tubular epithelium (renal lipidosis)

Kidney: Glomerulonephritis, diffuse, chronic, minimal



Liver, cynomolgus macaque. The liver is diffusely pale yellow with rounded edges. (Photo courtesy of: Wake Forest School of Medicine, Department of Pathology/Comparative Medicine, Medical Center Boulevard, Winston Salem, NC 27157-1040 http://www.wakehealth.edu/School/Comparative-Medicine/Training-Programs/ACVP.htm)

Contributor's Comment: Additional relevant microscopic findings included vacuolar hepatocellular degeneration (hepatic lipidosis) and necrosis of adipose tissue. Death was attributed to acute renal failure due to the massive accumulation of lipid and the associated metabolic derangements, all consistent with fatal fasting syndrome of obese macaques. The necropsy findings, along with the marked azotemia, hyperphosphatemia, advanced age, recent stressors and obesity with rapid weight loss are all consistent with this syndrome.[8]

Fatal fasting syndrome has been reported in African green monkeys (*Cercopithecus aethiops*), cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*), and shares similarities with conditions in other species, including hyperlipidemia in Shetland ponies, hepatic lipidosis in cats and guinea pigs, and pregnancy toxemia in ruminants.[2] Obese, female, aged macaques are particularly susceptible to this syndrome, which is often precipitated by a period of anorexia, weight loss, or stress.[2,3,7,8] The pathogenesis has not yet been fully elucidated, but a period of inadequate caloric intake in obese animals is a common theme among the disease profiles of the all the aforementioned species. Regardless of the initiating factor, an over-compensation of the liver results in fatty acid mobilization beyond the body's ability to metabolize, resulting in a metabolic imbalance with an inability to process the excessive triglycerides and lipoproteins.[4,7]

The most common associated clinical pathology finding is renal azotemia.[8] Hypertriglyceridemia is a common associated finding among the similar metabolic derangements across species. Triglycerides are not considered inherently toxic, but the products produced with triglyceride breakdown or failure of esterification (non-esterified fatty acids, ceramides, diacylglycerols) have detrimental effects on cells.[10] Lipotoxicity has been described in a several animal models throughout non-adipose tissues including cardiac and skeletal myocytes, hepatocytes, pancreatic B-cells and renal tubules.[9] Lipotoxic mechanisms leading to cellular dysfunction and injury may involve reactive oxygen species, intra-cellular pathway disruption, organellar damage, and lipid-induced apoptosis.[1] Renal tubular degeneration and detachment can lead to luminal tubule obstruction, increasing the interstitial pressure and ultimately reducing the glomerular filtration rate with subsequent acute renal failure.[4]

The reason for the female predisposition to this syndrome is unclear, but hyperlipidemia in Shetland ponies is similarly gender predisposed.[5] In cats with hepatic lipidosis, males and females are equally represented.[6]

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JPC Diagnosis: Kidney, proximal convoluted tubular epithelium: Lipidosis, diffuse, severe with mild tubular proteinosis.

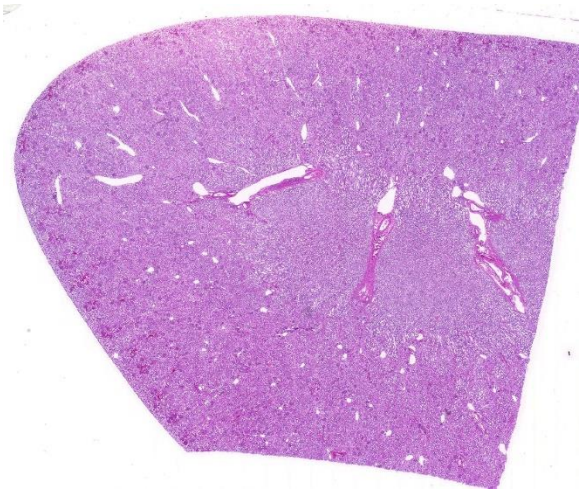
JPC Comment: The contributor has done an excellent job describing this classic metabolic condition in the macaque.

One of the obvious morphologic abnormalities in this section is the disproportionate amount of lipid within the cells of the proximal convoluted tubules. As the proximal convoluted tubules are the site of albumin resorption in the kidney, and free fatty acids (FSA) are normally complexed to albumin[1], this may explain the predominance of lipid within cells of the proximal convoluted tubules. Whether significant lipid synthesis from non-lipid substrates occurs in renal tubular epithelium and pathways of lipid export from the kidney are subjects of current conjecture and research.[1]

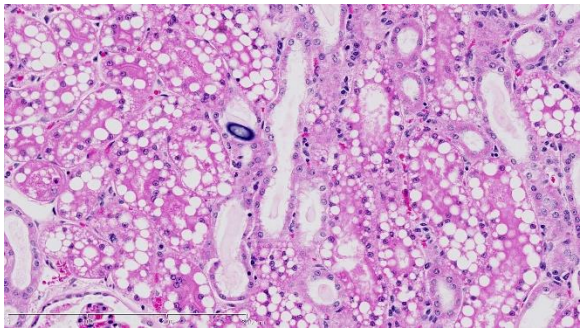
The link between lipidosis and kidney disease was first suggested more than 150 years ago by Rudolph Virchow, and the association between kidney disease and renal lipid accumulation has been demonstrated in the number of rodent models including mice on high fat diets, mice and rats with leptin deficiency, Type 1 diabetes and various transgenic models.[1] Lipotoxicity is usually accompanied by accumulation of neutral lipids in renal tubular epithelium and other cells as triglycerides. Triglycerides in themselves are non-toxic, with toxicity deriving mainly from long-chain non-esterified fatty acids (NEFA) and other breakdown products such as ceramides and diglycerols, and the association with NEFA-induced mitochondrial de-energization or persistent ATP depletion has been well-documented in renal reperfusion injury as well other forms of lipid-induced nephrotoxicity.[2]

Fatal fasting syndrome in macaques present in numbers of similarities to syndromes associated with stress, anorexia and lipidosis of multiple organs in both the cat and the horse. Hepatic lipidosis is a well-known metabolic condition in cats resulting from severe prolonged caloric and protein restriction. Carbohydrate restriction is the primary driver of mobilization of peripheral fat stores and flooding of the liver with NEFA. In the normal state, NEFA enter the mitochondria and are converted to energy. In excess, NEFA are either stored within the hepatocyte, or esterified with lipoproteins and re-secreted as VLDLs. In cats with hepatic lipidosis, concurrent protein starvation precludes production of lipoproteins and retention of massive amounts of triglycerides as lipid vacuoles within the hepatocyte.

In horses, hyperlipidemia is associated with



Kidney, cynomolgus macaque. A section of renal cortex and medulla is submitted for examination. There is mild diffuse pallor of the renal cortex. (HE, 6X)



Kidney, cynomolgus macaque. The renal cortical proximal tubular epithelium is diffusely vacuolated with occasional epithelial cell attenuation and nuclear pyknosis. Intraluminal mineral is present in some tubules. (Photo courtesy of: Wake Forest School of Medicine, Department of Pathology/Comparative Medicine, Medical Center Boulevard, Winston Salem, NC 27157-1040 http://www.wakehealth.edu/School/Comparative-Medicine/Training-Programs/ACVP.htm)

additional risk factors including breed (ponies, donkeys, and miniature breeds), gender (with mares accounting for between 75 and 100% of cases) and stress (more commonly associated with late pregnancy and early lactation). In ponies and donkeys, hyperlipidemias usually a primary disease process with stress and obesity appearing to be particularly important predisposing factors. Gross and histologic findings mirror similar syndromes in macaques and cats with gross lipidosis of multiple organs - hepatic lipidosis in affected animals may be severe enough to result in signs of colic from overstretching of the hepatic capsule.[5]

On a peripherally related subject, a recent article identified interstitial lipid accumulation as a long-term histologic findings in cats with chronic renal disease.[8] Data from the study suggests that the interstitial lipid accumulation may be the result of tubular epithelial degeneration and lysis as well as tubular basement membrane fragmentation in a species in which tubular lipidosis is often seen in the normal state. In the animals of the study, interstitial lipid was not equally distributed between the right and left kidneys, but all was found in the cortical regions. In light of recent research on mechanisms of renal lipoma toxicity, this is an interesting finding which may contribute to the overall progression of this common disease in cats.[8]

Within the seminar, there was discussion about the use of the term "vacuolar degeneration, lipid-type" versus "lipidosis" in the morphologic diagnosis for this particular lesion. There was general agreement that the former is better reserved for situations in which morphologic evidence of cellular degeneration is noted beyond simple lipid vacuolation or swelling. In this case, without either clinicopathologic evidence of tubular damage, or morphologic evidence of tubular damage, it was thought that lipidosis (or vacuolar change) would be most a more appropriate choice..

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CASE IV: 15-H700012 (JPC 4084546).

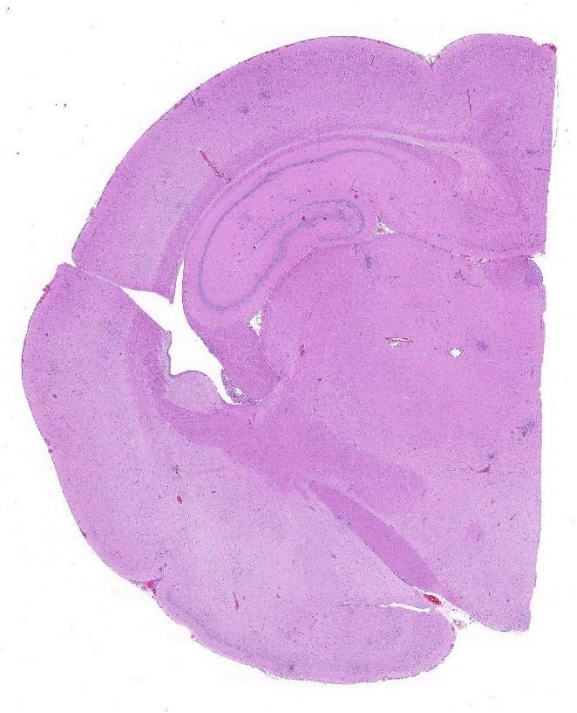
Signalment: 21 week old, intact male, domestic rabbit (*Oryctolagus cuniculus*)

History: 1 day history of sneezing with acute onset on respiratory distress.

Gross Pathology: The animal was in poor nutritional condition characterized by a near-complete lack of subcutaneous, perirenal and mesenteric adipose tissue. The right nasal cavity was nearly completely filled with a tan, viscous material.

Laboratory results: None given

Microscopic Description:
BRAIN (Some slides contain cerebrum while others contain hippocampus and



Cerebrum, rabbit. Inflammatory foci are visible within both grey and white matter at subgross magnification. (HE, 5X)

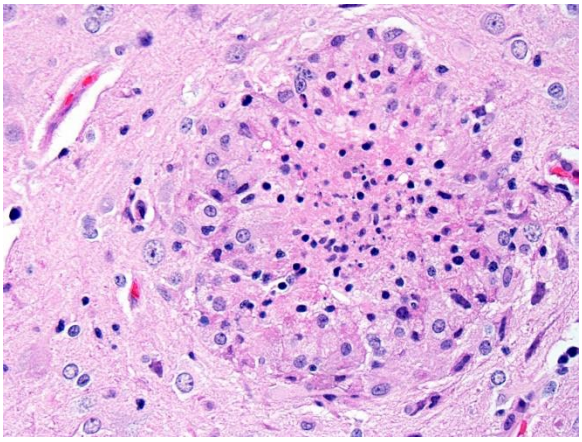
cerebellum, but the lesions are identical): Infiltrating both grey and white matter, there are multiple aggregates of epithelioid macrophages with lesser numbers of lymphocytes and plasma cells and rare heterophils. The inflammatory infiltrates frequently surround accumulations of eosinophilic cellular and karyorrhectic nuclear debris (necrosis). Occasional necrotic foci contain irregular, fragmented, basophilic material (mineral). Within inflammatory foci, there are rare, 20-30 µm pseudocysts that contain numerous 1x3 µm refractile spores with a small, basophilic nucleus. The neuropil surrounding the necrotic and inflammatory foci contains moderate numbers of reactive glial cells (gliosis). Vascular endothelial cells are frequently plump (reactive), and Virchow-Robin spaces often are expanded with macrophages, lymphocytes and plasma cells. The leptomeninges are expanded with moderate numbers of lymphocytes and plasma cells.

Contributor's Morphologic Diagnoses: Brain (cerebrum, hippocampus, and cerebellum): Meningoencephalitis, granulomatous and necrotizing, multifocal with microsporidial spores (*Encephalitozoon cuniculi*).

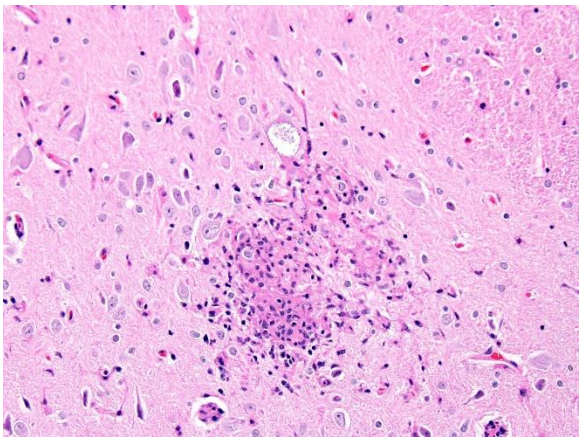
Contributor's Comment: Wright and Craighead initially identified *Encephalitozoon cuniculi* in 1922 as the agent responsible for "infectious motor paralysis" in rabbits exhibiting lethargy, tremors and paresis.[14] *E. cuniculi* are members of the phylum Microspora, which contain a diverse collection of obligate intracellular organisms which can exist as environmentally resistant spores outside of the host.[12] There has been considerable debate and reclassification with regards to the evolutionary origin of Microspora. These intracellular eukaryotes lack mitochondria and peroxisomes, and were originally thought to belong to a deeply branching protist lineage diverging prior to the emergence of the mitochondria as an endosymbiont (11). Modern phylogenetic analysis reveals that Microsporidia are actually more closely related to the fungal domain.[5] Phenotypically, the defining criterion for inclusion is the phylum is an organelle found coiled within the spore termed a "polar filament" or a "polar tube".[1]

Besides *E. cuniculi*, two other species with the genus *Encephalitozoon* are also known to infect mammals: *Encephalitozoon hellum* and *Encephalitozoon intestinalis*.[8] *E. cuniculi* is capable of infecting a wide range of mammalian hosts, including rabbits, rodents, horses, carnivores and humans.[7] In humans, *E. cuniculi* and other microsporidia have been identified as opportunistic pathogens in immunocompromised patients, though not causing the same epidemiological human morbidity and mortality as other parasites such as *Plasmodia, Trypanosoma, Leishmania* and *Toxoplasma gondii*. At one time, *E. cuniculi* was thought to be the agent responsible for rabies and polio.[12]

The main host for *E. cuniculi* is the rabbit,



Cerebrum, rabbit. Inflammatory foci are composed of a combination of activated glia and epithelioid macrophages which are centered on cellular debris (and likely Encephalitozoon spores.) (HE, 400X)



Cerebrum, rabbit. Cells with cytoplasmic parasitophorous vacuoles containing spores are present in close apposition to "granulomas". (HE, 400X)

and infections are mostly subclinical, and the course is typically chronic, taking weeks to months to develop a parasite burden sufficient to cause clinical signs.[7] The seroprevalence in pet populations is high (between 37% and 68%) due to proximity.[4] Historically, in laboratory rabbit colonies, *E. cuniculi* was a significant problem that resulted in interference with experiments and affected the overall health of the colonies.[10] Generally, infected rabbits will exhibit the non-specific signs of weight loss and failure to thrive, but can also manifest neurological signs such as ataxia, opisthosomas, torticollis, hyperesthesia, or paralysis.[6] Phacoclastic uveitis is also possible, and is characterized by a mixed inflammatory response (granulocytes, macrophages and multinucleate giant cells) causing lens capsule rupture, with organisms found only within the lens.[3]

In naturally infected rabbits, transmission occurs through organisms shed in the urine that are orally consumed, although transplacental infections are documented.[9,12] In experimental conditions, rabbits are also capable of being infected via a respiratory route in addition to oral. Following exposure, spores infect mononuclear cells and thereby enter they systemic circulation via leukocyte trafficking.[9] The first organs affected are the lung, liver and kidney, but later (approximately 3 months post-exposure), the lesions in the lung and liver subside and there are significant changes to the brain as well as the kidneys.[7] Gross lesions are not typically seen, although rarely there can be focal, irregular and depressed regions in the renal cortex.[2]

Reliable histological lesions in the brain consist of focal to multifocal, non-suppurative meningoencephalitis with gliosis and perivascular accumulations of lymphocytes and plasma cells. In some instances, there can be foci of necrosis surrounded by epithelioid macrophages with lesser numbers of lymphocytes and plasma cells.[13] Lesions in the kidneys include focal to segmental interstitial nephritis with tubular epithelial degeneration, necrosis and sloughing. There is little to no glomerular involvement.[9,13] Spores can be identified using Gram stain an ovoid, 1.5 x 2.5-5 um diameter structures, which also stain purple with carbol fuchsin.[9]

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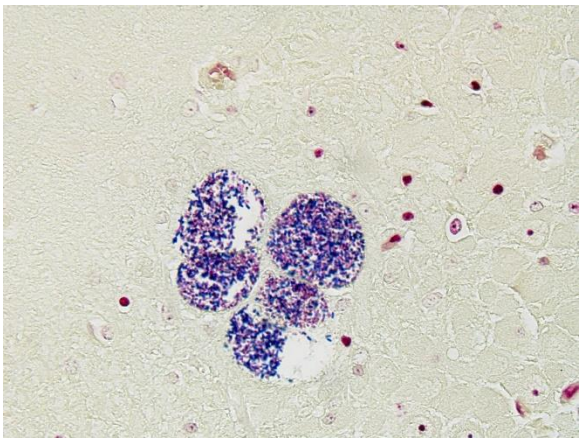
JPC Diagnosis: Cerebrum: Encephalitis, granulomatous and necrotizing, multifocal, moderate with lymphoplasmacytic meningitis and numerous microsporidian spores.

JPC Comment: Microsporidia are unique organisms which apparently have evolved from fungi, being most closely related to the

zygomycetes.[7] Other similarities between microsporidia and fungi include the presence of chitin and trehalose, similarities between the cell cycles, and the organization of certain genes. Other unique factors include their lack of mitochondria (although enzymes with mitochondrial functions have been conserved), and ribosomal RNA which more closely resembles that of prokaryotes than eukaryotes. Particularly impressive is their extremely small genome, comprising 2.9 Mbp, which has been accomplished due to the compression of genetic information within chromosomes, resulting from a virtual absence of introns, a low number of repetitive sequences and many single-copy genes, an overall shortening of genes as well as of non-coding sequences, as well as the absence of genetic information regarding certain metabolic pathways that are unnecessary for the organism's parasitic lifestyle.[7,8]

The unicellular spores of these parasites are unique within the animal kingdom with incorporation of the nucleus, the posterior vacuole, and ribosomes into "sporoplasm", which is injected into new host cells by virtue of a polar tube, a specialized invasion apparatus which works much like a hypodermic needle. In addition, the polar tube may stain positively with a number of histochemical stains, including modified trichrome, Luna, PAS, and Giemsa facilitating identification of microsporidia in tissue section.



Cerebrum, rabbit. Spores of Encephalitozoon within intact pseudocysts stain gram-variable (predominantly gram-positive) on tissue gram stains. (Brown-Hopps, 400X)

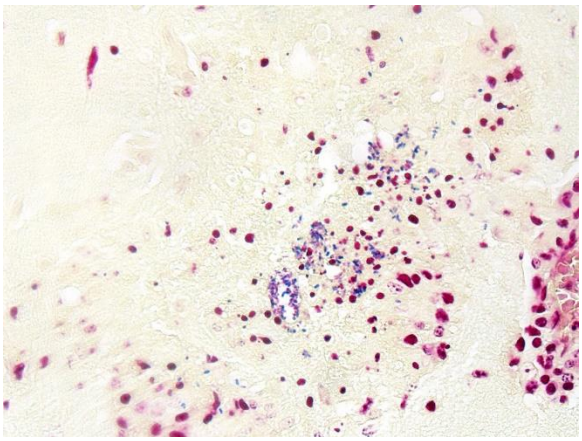
E. cuniculi may parasitize a wide range of mammals, but is most commonly associated with infections in rabbits, rodents, dogs, and primates. Based on the number of short repeats in the ribosomal internal transcribe spacer region, three different strains of *E. cuniculi* of have been identified: the "rabbit" (type I), the "mouse (type 2), and the "dog" stain (type 3). These strains are useful in identifying the source of human outbreaks (with only infections with dog and rabbit strains being previously identified). Outbreaks in rabbits appear to occur solely from rabbit strains.[8]

The contributor has an excellent job in detailing infection in the rabbit. While lesions in the rabbit are classically considered to be limited to the CNS and kidney, acute infections may results in cysts and spores within the lung, liver and heart, sites which are clear of organisms when the clinical signs of neurologic disease or renal failure become evident. Lenticular invasion is a particularly interesting phenomenon which appears to be restricted to dwarf rabbits; unlike normal horizontal infections, this syndrome results from vertical transmission to the developing lens via a peculiar homing mechanism for *E. cuniculi* to the developing optic cup where it takes residence within the lens. Delayed replication results in destruction of lens fibers and leaching of lens protein into the globe, ultimately causing severe granulomatous inflammation and phthisis.

Infections with *Encephalitozoon* sp. and a related microsporidian genus,

Enterocytozoon, have been reported in humans. While most cases of infection of *E. cuniculi* are associated with severe immunodeficiency, a number of cases have occurred in immunocompetent individuals in close contact with rabbits and dogs. As spores of E. cuniculi are resistant within the environment, waterborne infections could not be totally excluded in such cases.[8] Other related species of *Encephalitozoon*, to include *E. hellem* and *E. intestinalis*, are also well reported in the literature. *E. hellum*, is a microsporidian parasite primarily of psittacine birds which has been almost exclusively diagnosed in HIV-infected individuals. *E. intestinalis* is the second most prevalent microsporidian species infecting humans and is a common cause of diarrhea and other gastrointestinal complaints in HIV-infected individuals.[8]

A related microsporidia parasite, the most common species known to cause human disease, is *Enterocytozoon bienusi*. This pathogen was first described in HIV-infected patients in 1985 and may be found in up to 50% of HIV infected patients.[8] It has also been identified in immunocompetent patients associated with traveler's diarrhea in Europe. *E. bienusi* has also been identified in immunocompetent diarrhea patients in concert with *E. hellem* and *E. intestinalis*.[8] Over the last decade, evidence has accumulated that this parasite may persist as an asymptomatic infection in immunocompetent humans. Eleven years after its discovery as a human pathogen, *Enterocytozoon* was detected in animals for the first time and seems to be a common parasite in asymptomatic pigs.[8] It has also been reported as a common finding in SIV-infected macaques, but may also be seen in immunocompetent macaques as well. It has not been documented in macaques in the wild.[8]



Cerebrum, rabbit. Gram stains delineate extracellular spores within inflammatory foci which resemble cellular debris. (Brown-Hopps, 400X)

The determination of the morphologic diagnosis in this case was met with spirited discussion, and other descriptive features of the morphology of this disease were also greeted with some skepticism (including the oft-used term "pseudocyst"). Granulomatous encephalitis is a classic description of the inflammation associated with cerebral encephalitozoonosis (and is used in some very recent textbooks on laboratory animal disease, yet the true composition of the inflammatory foci surrounding extracellular spores is difficult to elucidate on morphologic grounds alone, and a more in-depth analysis of cell types contained in these foci is not available. The lack of multinucleated cells, lymphocytes, and plasma cells in inflammatory foci do not support granulomatous inflammation, and pathologists who refer to these foci as "glial nodules" may in fact be closer to the truth. Our morphologic diagnosis above is a combination of tradition and morphology – granulomatous on faith to excellent morphologists who preceded us, and necrotizing on evidence.

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