



WEDNESDAY SLIDE CONFERENCE 2019-2020

Conference 17

5 Feb 2020

CASE I: 1235813-006 (JPC 4117518).

Signalment: Three year old, male cynomolgus macaque (*Macaca fascicularis*) from China.

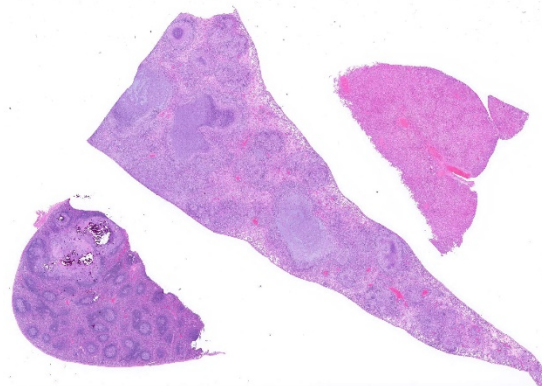
History: This animal was found dead on day 109 of a 6 month routine general toxicity study. There were no reported clinical signs. Routine infectious agent screens and three consecutive tuberculosis skin tests were negative with the exception of measles which was positive after a live vaccine.

Gross Pathology There were moderate, focal to multifocal, tan foci on the liver and lung with moderate swelling of the mediastinum. Skin discolorations with abrasions / scabs were present on the ventral abdomen and right forelimb.

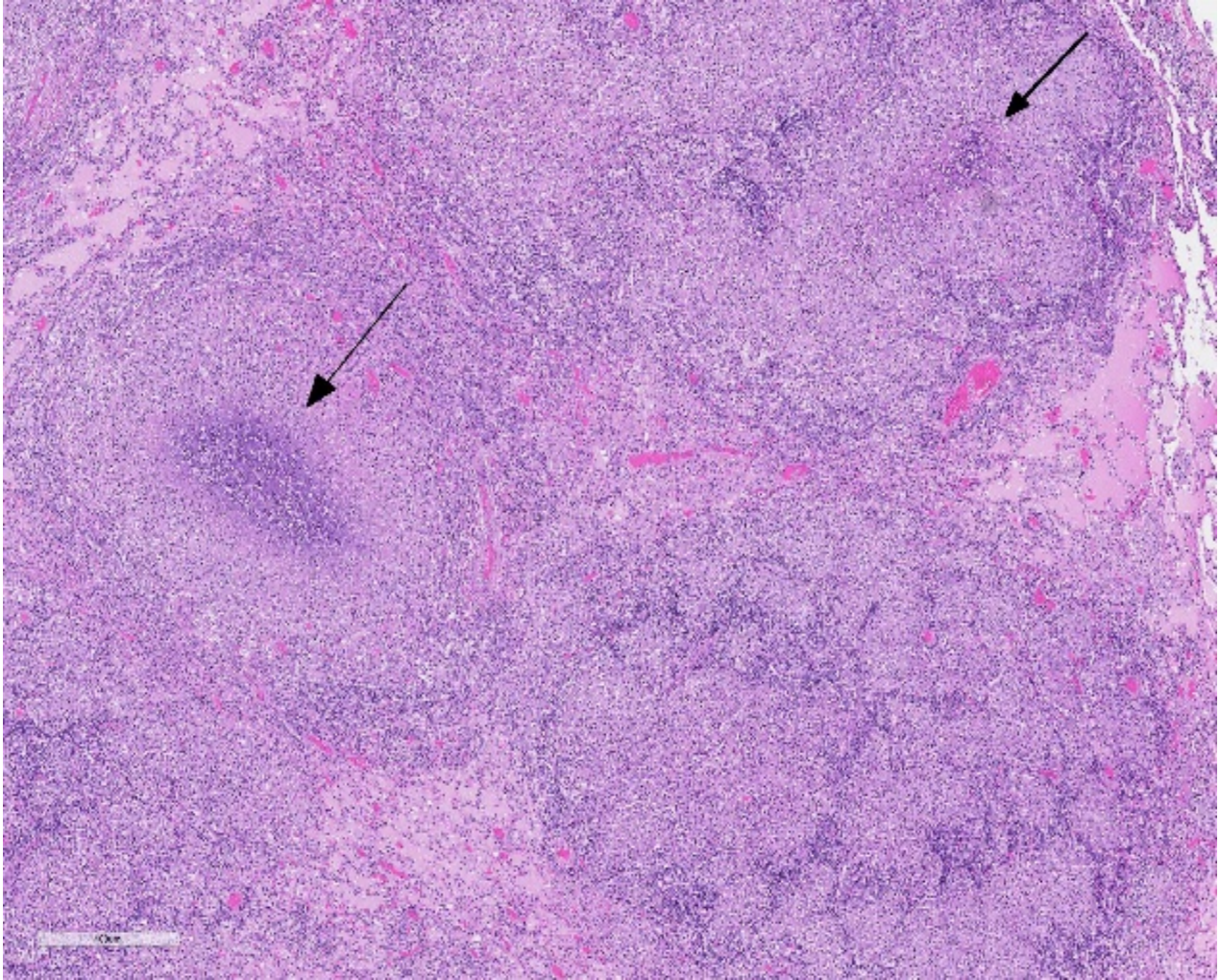
Laboratory results: The tan foci were PCR positive for *Mycobacterium tuberculosis* group and culture yielded slow

growing acid fast bacilli identified as *Mycobacterium tuberculosis*.

Microscopic Description: Throughout approximately 90% of examined lung there was multifocal to coalescing granulomatous inflammation often forming discrete granulomas with central eosinophilic flocculent debris (caseating necrosis) with or without basophilic granular mineralization. This central area was surrounded by



Liver, lung, spleen, cynomolgus monkey: Granulomas are easily visualized in the lung (middle) and spleen (bottom left) at subgross magnification. The granulomas in the spleen are heavily mineralized. (HE, 5X)



Lung, cynomolgus monkey. There are many areas of coalescing granulomatous inflammation in the lung. More mature granulomas are centered on an area of necrotic debris. (HE, 53X

epithelioid macrophages, multinucleated giant cells, a rim of lymphocytes and variable fibroblasts/fibrosis. Similar solitary discrete granulomas were seen in the spleen, liver, and mediastinum (mediastinum not submitted). There was an increase in number and prominence of germinal centers in the spleen (follicular hyperplasia) as well as frequent multifocal type 2 pneumocyte hyperplasia in the lung.

Ziehl-Nielsen acid fast staining of the lung revealed rare acid fast bacilli within the cytoplasm of macrophages or multinucleated

giant cells, and/or free within the necrotic/mineralized areas.

Contributor’s Morphologic Diagnosis:

Granulomatous pneumonia, severe, diffuse with focal hepatic and splenic granulomas and scant acid-fast bacilli.

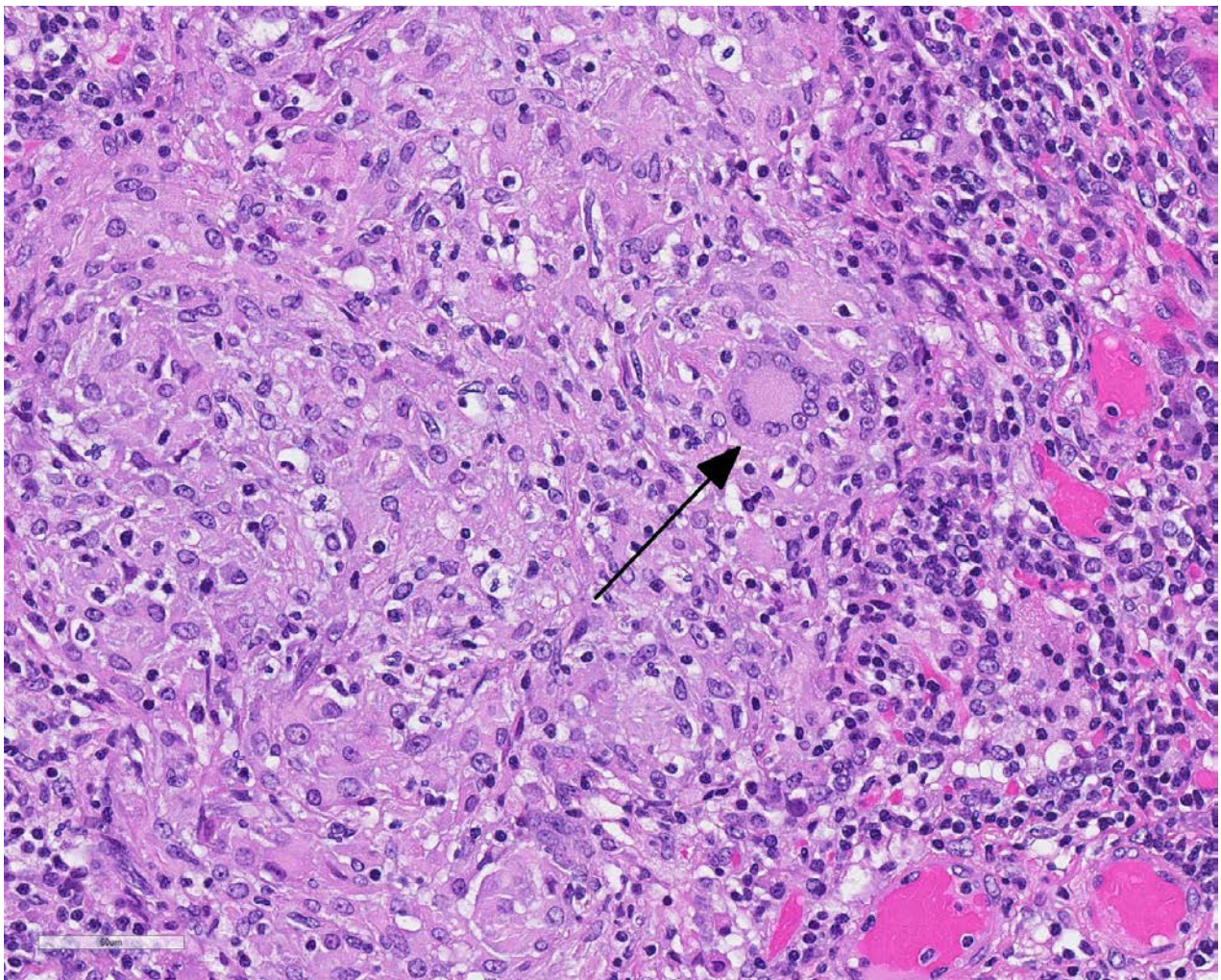
Contributor’s Comment:

Mycobacterium tuberculosis (M.tb) is a cosmopolitan organism that infects approximately 1/3 of the world’s human population and is a leading cause of death due to infectious disease. It is also an increasing public health

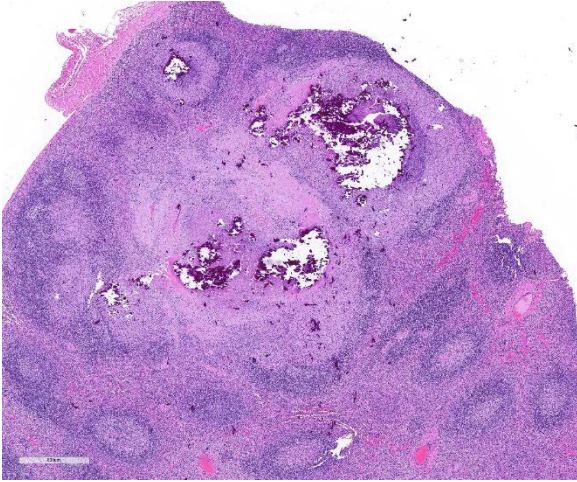
concern due to 1) emerging multidrug resistant and extensively drug resistant *M.tb* and 2) the increasing risk of death in HIV infected people.²⁰ Tuberculosis was considered the cause of 40% of all HIV-related deaths in 2016.²⁰ Roughly 10% of people infected with *M.tb* go on to develop active contagious disease with the remaining individuals either clearing the bacteria (approximately 10%) or harboring latent disease. These latent carriers create a reservoir of tuberculosis which can reactivate during times of stress or immune suppression and disseminate *M.tb* to naïve

populations of humans²⁰ or spread the disease to susceptible animals (reverse zoonosis).¹³

In an NHP necropsy, *M.tb* often presents as tan or creamy white nodules (granulomas), predominantly within the lung, but also with systemic involvement. Other common causes of tan nodules or granulomas within the lung includes foreign body (kaolin), protozoan (*Toxoplasma gondii*), bacterial (*Nocardia* sp), mycotic (*Coccidioides immitis*, *Coccidioides posadasii*, *Histoplasma capsulatum*, *Blastomyces*



Lung, cynomolgus monkey. There are many areas of coalescing granulomatous inflammation in the lung. More mature granulomas are centered on an area of necrotic debris. (HE, 53X)



Spleen, cynomolgus monkey. Similar granulomas are present at one end of the section of spleen, but with a high content of crystalline mineral in the center. There is marked follicular hyperplasia of the white pulp through the remainder of the section, and the normal sinusoidal architecture is effaced by marked reticuloendothelial hyperplasia. . (HE, 331X)

dermatitidis, and *Cryptococcus neoformans*) and parasitic organisms (*Pneumonyssus simicola* (acariasis), *Taenia* cysts, *Echinococcus* cysts, *Mesocestoides* tetrathyridia, *Spirometra* spargana, and *Filaroides* and *Filariopsis* nematodiasis), Histology can distinguish between these diagnoses based on the appearance of the organisms.¹¹

M.tb and mycobacteria in general are unusual among bacteria due to the high lipid content and relative thickness of their cell wall compared to gram-positive and gram-negative bacteria. The thick mycobacterial cell wall is relatively impermeable, does not take up crystal violet (Gram stain variable), and is responsible for the acid fast staining properties of mycobacteria.

Immunologically, the lipid component of the mycobacterial cell wall is particularly effective at recruiting an immune response and has been commonly used as an adjuvant

in vaccines to recruit a desired robust immune response to antigens of interest. The protein (antigen) and lipid components together during mycobacterial infection result in sensitization of the animal to tuberculosis proteins. Sensitized animals then react to purified mycobacterial protein derivative (PPD) in a skin test with a Type IV hypersensitivity reaction resulting in a cell mediated immune reaction after 2-3 days. Non-responders, or animals that fail to demonstrate a positive response to the PPD skin test in the face of mycobacterial infection (as seen in this monkey) are thought to be due to immunologic anergy.³ Anergy is a failure of the animal to produce a Type IV delayed type hypersensitivity reaction upon PPD exposure. The exact cause of anergy in *M.tb* infection is unknown; although, in general, it is a lack of response of cell mediated immunity due to tyrosine kinase blockage and reduced IL-2 receptor signaling, among other mechanisms.¹⁷ In *M.tb* infection a weak cell-mediated immune response (high IL-4) is thought to be associated with anergy.⁷

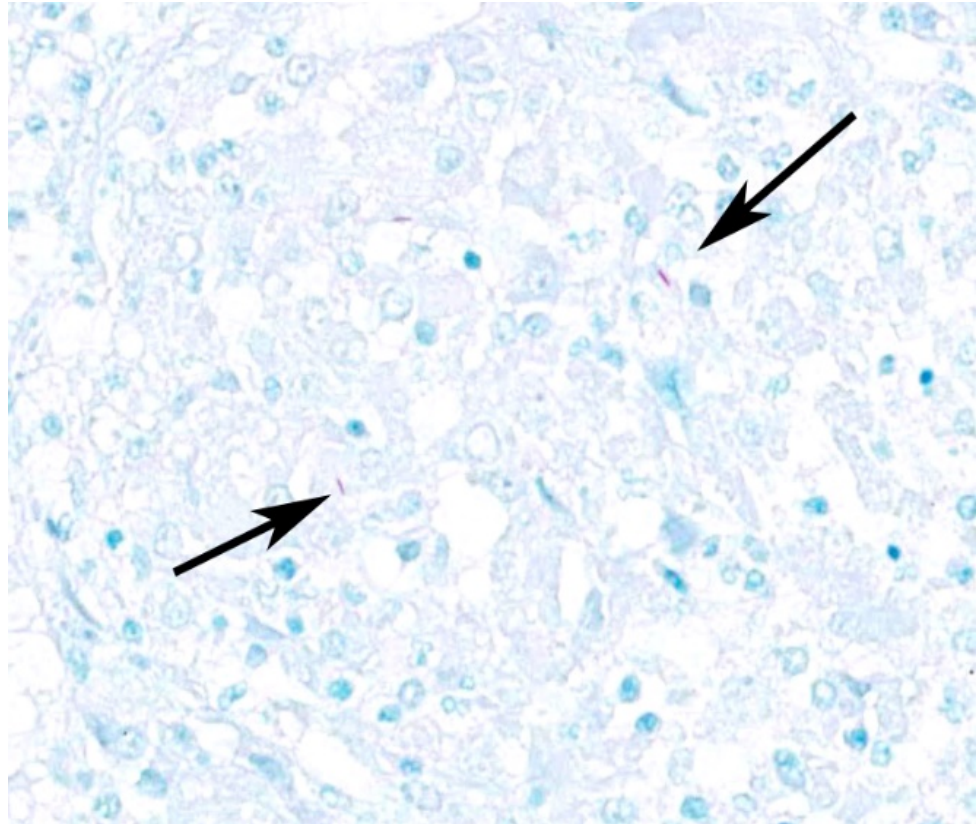
Another feature of the mycobacterial cell wall is its role in allowing the bacteria to evade the host immune system. In part, the success of *M.tb* is due to the highly infectious nature of the bacteria. It is thought that as few as 10 bacteria can cause disease.¹⁸ Once within the host, mycobacteria are engulfed by macrophages into phagosomes. Under normal circumstances within a macrophage, phagosomes mature and fuse with lysosomes exposing the bacteria to destructive chemicals and lytic enzymes leading to bacterial death. Mycobacteria

have evolved mechanisms to avoid phagosome-lysosome fusion and survive within macrophages. For example, components of the mycobacterial cell wall interrupt the normal traffic of the phagosome to the lysosome by preventing recruitment of key molecules in phagosomal maturation such as the Rab5 to Rab7 conversion.¹

Phagosome maturation arrest in *M.tb* is thought to

involve specific cell surface receptors (i.e. mannose receptor) and mycobacterial cell wall components (i.e. lipoarabinomannan and SapM enzyme)⁵. Ultimately, whether the mycobacteria are destroyed or remain active or latent within the host depends upon the infectious dose, the host immune response, and the environment of the infection.³ A host response characterized by a Th2 cytokine response (IL-4) is associated with mycobacteria infection and Th1 cytokine response (IL-12, TNF- α , and IFN- γ) is associated with bacterial clearance.¹

There are many mycobacteria that cause significant human and animal diseases. The histologic features of these diseases range from structured granulomas (often



Lung, cynomolgus monkey. Acid-fast bacilli (arrow) are rarely seen within the granulomas. (Ziehl-Nielsen, 400X)(Image courtesy of: MPI Research, a Charles River Company, <https://www.mpiresearch.com>)

associated with few bacteria/paucibacillary infection) to more diffuse macrophage infiltration (often associated with numerous bacteria/multibacillary infection).

Granulomas, or tubercles, have four histologic features: 1) central caseous necrosis with or without mineralization, 2) surrounded by epithelioid macrophages and Langhans multinucleated giant cells, 3) surrounded by lymphocytes with or without neutrophils and peripheral fibrosis, 4) tubercles contain rare acid fast bacilli which are found within macrophages, multinucleated giant cells, and/or the caseous core. In paucibacillary mycobacterial diseases, gross observations are crucial to identifying areas to look for

these rare bacilli as bacteria are not typically found outside of the structured granulomas.³ Multibacillary infection is more variable with few to sheets of infiltrating macrophages containing few to numerous acid fast bacilli. Leprosy (*Mycobacterium leprae*) is centered on nerves and can be paucibacillary and granulomatous or multibacillary and diffuse depending upon the strength of the cell mediated immune response with a stronger cell mediated response associated with the paucibacillary infection.⁷ Common mycobacterial species involved in disease are listed in Table 1.

Contributing Institution:

MPI Research; a Charles River company

<https://www.crl.com>

<https://www.mpiresearch.com>

JPC Diagnosis:

1. Lung: Bronchopneumonia, granulomatous, multifocal to coalescing, severe.
2. Spleen: Splenitis, granulomatous, multifocal to coalescing, severe, with mineralization.
3. Liver: Hepatitis, granulomatous, multifocal, moderate.

JPC Comment: The contributor has provided an excellent review of infection by *M tuberculosis*, (which has also been a popular submission in non-human primates as well as various models over the years).

The very early history of *Mtb* is still conjecture, but early strains may have infected hominids up to three million years ago with modern strains originating some 15,000-20,000 years ago. Egyptian mummies have demonstrated skeletal

deformities reminiscent of “Pott’s disease”, and are even present in Egyptian art. The first written documents about TB appeared in India and China dating back to 3000 to 2000 years ago.²

TB was well known and referred to as *phthisis* in ancient Greece. Isocrates first suggested the contagious nature of “the king’s evil”. The Romans recognized the first symptomatology of TB and recommended fresh air, milk, and sea voyages as treatment².

Pulmonary TB was well known in Middle Ages Europe, with scrofula, a form of TB affecting the cervical nodes, described as a new clinical form. Before the first surgical interventions in the removal of “scrofula”, the touch of English or French royalty was considered a cure (and continued in France until 1825).²

The true infectious nature of “phthisis” and “consumption” was expounded upon by English physician Benjamin Martin, and the disease was first called “tuberculosis” Johan Schonlein in the mid 19th century. The industrial revolution and poorly ventilated and overcrowded workplaces and housing with poor sanitation resulting in a flourishing of TB, with up to 15% of workers dying of tuberculosis in 1838-1839 in England alone. The anemic plor of the afflicted resulted in another name – “the white plague”. The “white plague” reached a zenith in the mid-1800s, resulting in almost one in four deaths in Europe and North America².

The 1800s also brought a new and revised view of tuberculosis, with numerous renowned clinicians connecting the dots between miliary disseminated disease, tubercles, consolidation, pleurisy and pulmonary cavitation. In 1843, German

Mycobacteria	Species Affected	Systems involved and/or Gross appearance	Typical histologic appearance
<i>M. tuberculosis</i>	<p>humans, NHPs, elephants, psittacine birds (zoonosis and reverse zoonosis)</p> <p>exotic ungulates, marine mammals, carnivores¹⁴, dogs, cats, pigs, cattle</p> <p>Potential reservoir species: humans</p>	Granulomas – lung and systemic (i.e. liver, spleen, etc.)	granulomas
<i>M. bovis</i>	<p>cattle, dogs/cats, humans, cervids, swine, horses</p> <p>deer, elk, bison, buffalo, goats, camels, llamas, swine, elephants, rhinoceroses, dogs, foxes, cats, mink, badgers, humans, NHPs, pigs, goats, warthogs, cats, mustelids, humans</p> <p>Potential reservoir species: badgers, brush tailed possums, swamp buffalo, buffalo, bison, various species of deer</p>	<p>cattle: granulomas – lung and systemic (lymph nodes, gastrointestinal tract, etc)</p> <p>dogs and cats: skin nodules or thickening/inflammation</p> <p>humans: pulmonary granulomas or gastrointestinal inflammation</p> <p>cervids: pulmonary granulomas, superficial lymphadenitis with draining tracts (DDx: <i>Fusobacterium necrophorum</i>)</p> <p>horses: Gastrointestinal thickening/ granulomas</p> <p>swine: systemic granulomas</p>	<p>granulomas</p> <p>dogs and cats: unstructured pyogranulomatous inflammation of the skin dissecting along facial planes. (Note: central necrosis and paucibacillary help to distinguish from feline leprosy); often more granulation tissue with few scattered macrophages.</p>
<i>M. avium</i> subspecies <i>avium</i> and <i>hominissuis</i> ⁶	birds > swine, cattle, horses, sheep, NHPs, humans	<p>birds: thin body condition with intestinal and hepatic > systemic granulomas (lungs, air sacs, spleen, bone marrow and skin)</p> <p>humans: lymphadenitis, granulomas – lung and/or systemic</p>	granulomas
<i>M. avium paratuberculosis</i>	cattle, goats, sheep	<p>muscle atrophy/wasting; thickened mucosa of the ileum, large intestine, enlarged draining nodes; intimal plaques/mineral in thoracic aorta</p> <p>small ruminants: granulomas (enteric or vascular/lymphatic)</p>	<p>multibacillary – unstructured granulomatous enteritis with villus atrophy</p> <p>sheep: lepromatous neuritis reported</p>
<i>M. microti</i> ¹⁸	voles, humans	<p>voles: gastrointestinal granulomas</p> <p>humans: pulmonary granulomas</p>	granulomas
<i>M. africanum</i> ⁴	humans	Pulmonary granulomas	granulomas

<i>M. avium intracellulare</i> complex (MAC)	dogs, cats	atypical mycobacterial skin infection - single to multiple cutaneous and subcutaneous nodules, plaques, macules or diffuse swelling In cats: thickened, hardened tissue in the inguinal fat	multibacillary unstructured pyogranulomatous dermatitis and panniculitis. Can resemble feline leprosy. Occasionally fibroplasia may be marked and the lesion may appear as a spindle cell neoplasm
<i>M. marinum</i> ¹⁰	fish humans	fish: systemic disease (potential route of infection skin trauma or gastrointestinal tract) humans: skin granulomas > pulmonary granulomas	granulomas
<i>M. kansasii</i>	humans	cervical lymphadenitis; pulmonary granulomas	granulomas
<i>M. scrofulaceum</i> ¹⁵	humans	cervical lymphadenitis “scrofula”	granulomas
saprophytic Mycobacteria (<i>M. smegmatis</i> , <i>M. fortuitum</i> , <i>M. chelonae</i> ⁸ , <i>M. phlei</i> , <i>M. thermoresistibile</i> , <i>M. xenopi</i>)	dogs/cats	Focal thickened skin / inflammation	atypical mycobacterial skin infection - Similar to MAC although paucibacillary and clear vacuoles surrounded by neutrophils and peripheral epithelioid macrophages. Organisms can be found within the clear vacuoles
<i>M. leprae</i> ⁹	armadillos, sooty mangabeys, chimpanzees, cynomolgus macaques, red squirrels	Multifocal to coalescing granulomatous dermatitis with loss of sensation/body parts on face, hands, and feet.	granulomas (tuberculoid) to unstructured (lepromatoid) granulomatous inflammation centered on nerves
<i>M. lepraemurium</i>	rodents, cats	often head and limb cutaneous or subcutaneous nodules with or without alopecia or ulceration, often 1-3 year old cats	granulomatous dermatitis and/or panniculitis – large, pale, foamy macrophages; tuberculoid (organized granulomas) to lepromatous (sheets of cells); nerve involvement is only sporadic (distinct from human leprosy)
<i>Mycobacterium?</i>	Cats	Feline tuberculosis syndrome in adult cats with firm skin nodules with draining tracts	pyogranulomatous dermatitis and panniculitis
<i>Mycobacterium?</i>	Dogs	canine leproid granuloma syndrome – firm painless nodules mainly on the head and dorsal pinnae of short coated breeds	acid fast bacteria identified, but not cultured; histo description unavailable

Table 1. Common mycobacterial infection in nonhuman primates.

physician Philipp Klencke was able to reproduce the human and bovine disease in rabbits. In 1850, the “sanatorium cure” was described for the first time by Hermann

Berhmer, a botany student suffering from TB himself, and was used for the next 100 years as a mainstay of treatment for TB patients.²

In 1882, Robert Koch identified, isolated, and for the first time visualized the tubercle bacillus of *Mycobacterium tuberculosis* in a

Following on his work, Paul Ehrlich developed alternate methods of staining, which in the hands of Franz Ziehl and Friedrich Neelson, became the first acid alcohol method for demonstrating the bacillus in tissue.¹²

In 1895, Wilhelm Konrad von Rontgen discovered the use of X-rays and became the first physician to visualize and detect TB lesions in the lungs; X-rays were an inexpensive method of diagnostic screening together with the tuberculin skin sensitivity test until the 1950s. In 1890 Koch himself developed a concentrated filtrate of cultured tubercle bacilli which he called “tuberculin” or “Koch’s lymph” which he believed, could actually cure the disease. However, when injected, it caused violent reactions, high fever, expansion of pre-existing lesions, and a severe reaction at the site, which he noted on self-inoculation. While it never did cure the disease, it was noted that infected individuals would develop “allergy” upon injection, resulting in an effective diagnostic method. The tuberculin and its method of use in the skin continued to be refined until the development of tuberculin purified protein derivative (PPD) by American biochemists Florence Seibert and Esmond Long in 1930, which was able to also detect latent subclinical infections.¹²

Vaccination for TB over the years has been problematic at worst, ineffective most of the time, and today is used only in areas with a high prevalence of childhood disease and in people in the military and healthcare with a high risk of exposure. The most successful advance in vaccination came in 1905 by two French bacteriologists, Albert Calmette and Camille Guérin at the Pasteur Institute, who

number of manifestations of tuberculosis, including pulmonary, scrofulaceous, extra-pulmonary, and meningeal lesions

developed a live attenuated vaccine from a non-virulent strain. Over 100,000 children were vaccinated with this strain in the 1930s across Europe. Unfortunately 250 children were vaccinated with a BCG vaccine accidentally contaminated with a virulent strain in Lubeck Germany; 73 died and 135 developed clinical tuberculosis. Childhood vaccination continued in post-WWII Europe and Asia to combat a resurgence of TB, but ultimately was discontinued in the 1970s, when strict control programs were considered sufficient to control the spread.¹²

In 1993, the World Health Organization declared TB a global emergency, and in 1995 defined the directly observed treatment, short-course strategy (DOTS) control policy, which has decreased the global incidence of TB by 1.5% each year, and overall mortality from TB has declined 22% from 2000-2015. With new and effective anti-tuberculosis chemotherapeutic agents, the strategy of early diagnosis and targeted therapy is now considered to have the best cost/benefit ratio for treating this worldwide disease.¹²

The moderator and attendees reviewed a number of genpath concepts regarding mycobacterial infections, a number of species and concepts regarding mycobacterial infections in a variety of species, and some diseases that might grossly cause nodules in the lungs of non-human primates. She mentioned the current theory of aortic mineralization in cattle with severe granulomatous disease as being the result of production of 1,25-cholecalciferol by the large numbers of macrophages in this disease and hypercalcemia.

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CASE II: WSC 2013 Case 1 (JPC 4032584).

Signalment: 119.2 kg (262.8 lb), 5-month-old, female, Hereford calf (*Bos primigenus*)

History: The day prior to euthanasia, the calf was found struggling to walk, and within 4 hours was recumbent. Four days previously, another 6-month-old heifer calf was found dead on this farm.

Gross Pathology: Body condition was good with visible subcutaneous and visceral fat. There was mild interlobular septal edema in the lungs and a large amount of blood exuded from the cut surface. Few pinpoint to 3mm diameter white foci were present both on the surface and within the parenchyma of the liver. There was mild enlargement of the spleen (interpreted as a

result of euthanasia). The serosa of the terminal segment of the ileum was reddened and the contents were watery and red. The urinary bladder contained a moderate amount of clear urine. Normally formed feces were present in the rectum.

Laboratory results: Immunofluorescence Test for Rabies Virus Antigen: Positive

Microscopic Description: Medulla oblongata: Multifocally, blood vessels are surrounded by 1 to 6 cell thick cuffs of lymphocytes with lesser numbers of macrophages and plasma cells. There are occasional mild perivascular hemorrhages with edema. Increased numbers of glial cells, including astrocytes, microglia and oligodendrocytes, are present in the neuropil. Low numbers of neurons in areas of gliosis have one or multiple 2-4 um diameter, round to oval, eosinophilic, cytoplasmic inclusions (Negri bodies).

Contributor's Morphologic Diagnosis:

Medulla oblongata: Encephalitis, lymphocytic, plasmacytic and histiocytic, multifocal, moderate, with gliosis and neuronal intracytoplasmic inclusions (Negri bodies), etiology consistent with rabies virus (RABV)

Contributor's Comment: The microscopic and immunofluorescence findings in this calf indicate rabies encephalitis. Rabies virus (RABV) is a lyssavirus in the family Rhabdoviridae that causes acute, progressive encephalitis that is almost invariably fatal.⁶ Two biotypes of RABV exist, i.e. "fixed" virus and "street" virus.³ Fixed RABV is the

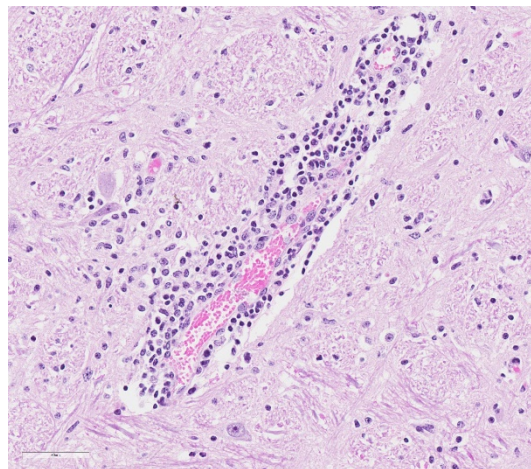


Brainstem, 5-month-old calf. One cross section of the brainstem is presented for examination. There are no apparent lesions at subgross magnification. (HE, 6X)

stabilized laboratory biotype, which is highly neurotropic but does not create Negri bodies and is not secreted in saliva, while street RABV is the wild biotype that circulates in nature.³ All mammalian species can be infected by RABV, which is most commonly transmitted by bites of carnivores or bats. The virus can also enter via sensory nerve endings of the skin and mucous membranes, which has been observed in rare instances of aerosol exposure and ingestion (e.g. cannibalism and scavenging).⁵ RABV infection has been documented after human organ transplantation, e.g. cornea, kidney, pancreas and liver.⁵ The principal reservoir vector species within the USA and Canada vary by geographic region. Skunks, foxes and raccoons are important reservoir vectors in the continental United States and Canada, with raccoons of particular importance along the Atlantic coast, coyotes in southern Texas, and arctic foxes in Alaska.^{3,4} In central and South America, dogs are the most important reservoir vector, with rabies also reported in terrestrial wildlife, e.g. monkeys, wolves, skunks and foxes. The

Indian mongoose (*Herpestidae auro-punctatus*) has become an important vector species in the Caribbean after its introduction to control rodents.⁵ In Europe, the red fox is the principal reservoir vector, although dogs and the raccoon dog (*Nyctereutes procyonoides*) in Eastern Europe may serve as independent reservoir species or spill-over hosts dependent on the fox rabies cycle.⁵ In Africa and Asia, dogs are important reservoirs with spillover to other species a major problem, for example, rabies accounts for 25,000-30,000 human deaths annually in India⁸ and threatens the endangered Ethiopian wolf (*Canis simensis*) and the African wild dog (*Lycaon pictus*). Australia, New Zealand, Japan and the British Isles are free of RABV infection, but other lyssavirus species exist in bats in at least some of these countries.⁵ Fructivorous, insectivorous and vampire bats are capable of transmitting RABV.^{3,4}

The bite of a rabid animal delivers the virus into the striated muscle and connective



Brainstem, 5-month-old calf. Multifocally within the brainstem, Virchow-Robin spaces are expanded by 4-5 layers of lymphocytes and fewer histiocytes. (HE, 108X)

tissue at the site of inoculation. At the site of entry, the virus replicates for a short period in myocytes, then buds from the sarcolemma to invade the neuromuscular junction through conjugation with nicotinic acetylcholine receptors. Virus is then taken up at the axon terminus and delivered by retrograde axoplasmic flow within neurons to spinal cord and brain.^{3,4} Subsequent replication occurs in the limbic system and neocortex.^{3,4} The surface-exposed viral coat glycoprotein (RVG) is responsible for the neurotropism of RABV by binding to several neural tissue receptor molecules, specifically the neuronal cell adhesion molecule (NCAM) and the p75 neurotrophin receptor (p75NTR).³ In animal species that serve as vectors, the viral genome moves centrifugally from the central nervous system through peripheral nerves to a variety of tissues including the adrenal medulla, nasal mucosa, cornea, epidermis and most importantly, the salivary glands.^{1,3} In the salivary gland of these hosts, virions bud from plasma membranes at the apical (luminal) surface of mucous cells and into the intercellular canaliculi, and are released in high concentrations into the saliva resulting in highly infectious saliva.^{2,3,4}

Following a 1-8 week incubation period, the clinical course of rabies is usually acute, lasting 1-2 days, but occasionally lasting up to 14 days. Following a prodromal phase, aberrant behavioral patterns occur in the infected animal. There are two recognized clinical forms of disease: furious and dumb or paralytic.³ Clinical features of the furious form include restlessness, nervousness, aggression, inability to swallow water, hypersalivation, exaggerated response to

light and sound, and hyperesthesia. Following the furious phase, paralysis and recumbency ensues. Terminally, there are often convulsive seizures, coma and then respiratory arrest.^{2,3,4}

Gross anatomical lesions specific to RABV infection are typically not evident.

Histological lesions of rabies are those typical of nonsuppurative encephalomyelitis with ganglioneuritis. Inflammatory and degenerative changes are usually most severe from the pons to the hypothalamus and in the cervical spinal cord, with relative sparing of the medulla oblongata.³ The inflammatory reaction typically is one of perivascular cuffing and focal gliosis, although inflammatory changes may be minimal or absent in some cases.³

Intracytoplasmic viral inclusions, known as Negri bodies, are present most commonly in the hippocampus of carnivores and in cerebellar Purkinje cells of herbivores. Negri bodies are found in neurons that are otherwise histologically normal and are reported to be scarce in regions where inflammation is the most severe.³ The most severe lesions are commonly found in dogs, whereas other species (particularly ruminants, which are highly susceptible) may have minimal perivascular cuffing and few glial nodules in spite of having numerous Negri bodies.³ In ruminants, gliosis is prominent in both the white and gray matter. Perivascular cuffs are composed mostly of lymphocytes, and a ring of hemorrhage largely confined to the perivascular space is commonly found surrounding cuffed vessels.

Contributing Institution:

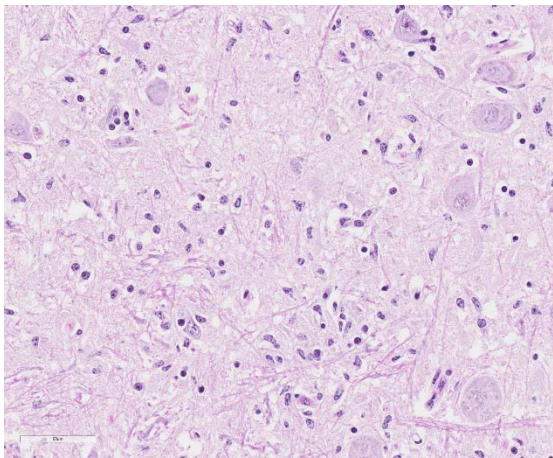
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JPC Diagnosis:

Brainstem: Meningitis, lymphocytic, diffuse, mild, with gliosis and occasional neuronal intracytoplasmic viral inclusions.

JPC Comment: As in Case 1 of this conference, a thorough and wide-ranging review by the contributor allows us to discuss some of the interesting aspects of the medical history of this well-known and global disease. The etymology of the word rabies itself is controversial, with authorities split on whether it derived from the word *rabias*, meaning “rabies” in Latin or the Sanskrit terms *rabhas* or *rabhasa*.⁷

Rabies has likely been known to humans ever since the domestication of dogs, estimated at up to 30,000 years ago. The first actual mention of rabies (and penalties



Brainstem, 5-month-old calf. There is a mild to moderate gliosis within brainstem nuclei, and occasionally poorly formed glial nodules (arrows). (HE, 400X)

for owners of rabid dogs) were found in Sumerian tablets dating back to 1930 BC – “If a dog becomes rabid [...] and the dog bites a man and causes his death, the owner of the dog shall pay forty shekels of silver; if it bites a slave and causes his death, he shall pay fifteen shekels of silver”. At this time, herbs were often used as first attempts to cure the disease, and failing that, incantations at the local temple. Dogs were thought to be more likely to become rabid during a lunar eclipse, particularly if it occurred at the year’s end.⁷

Many cultures in antiquity made attempts to prevent the transmission of disease. In Persia, cutting off a dog’s tail when they are 40 days old was done to protect them from the bite of another rabid animal. One prevention mistake in China during the Jin dynasty was to treat by applying the biting dog’s brain tissue to the bite wound to prevent disease.” Peditanus Dioscorides, a Roman physician living in Turkey may have started down a more appropriate path, calling for the heat cauterization of bites by rabid dogs, but this was later denounced by other Roman doctors as unnecessary brutal.⁷

In ancient Greece, many medical texts showed an increasing awareness of rabies and its effects, with detailed recunts of its symptoms in man and animals, recognition of a latency period between the bite and onset of symptoms, and even palliative care.⁷

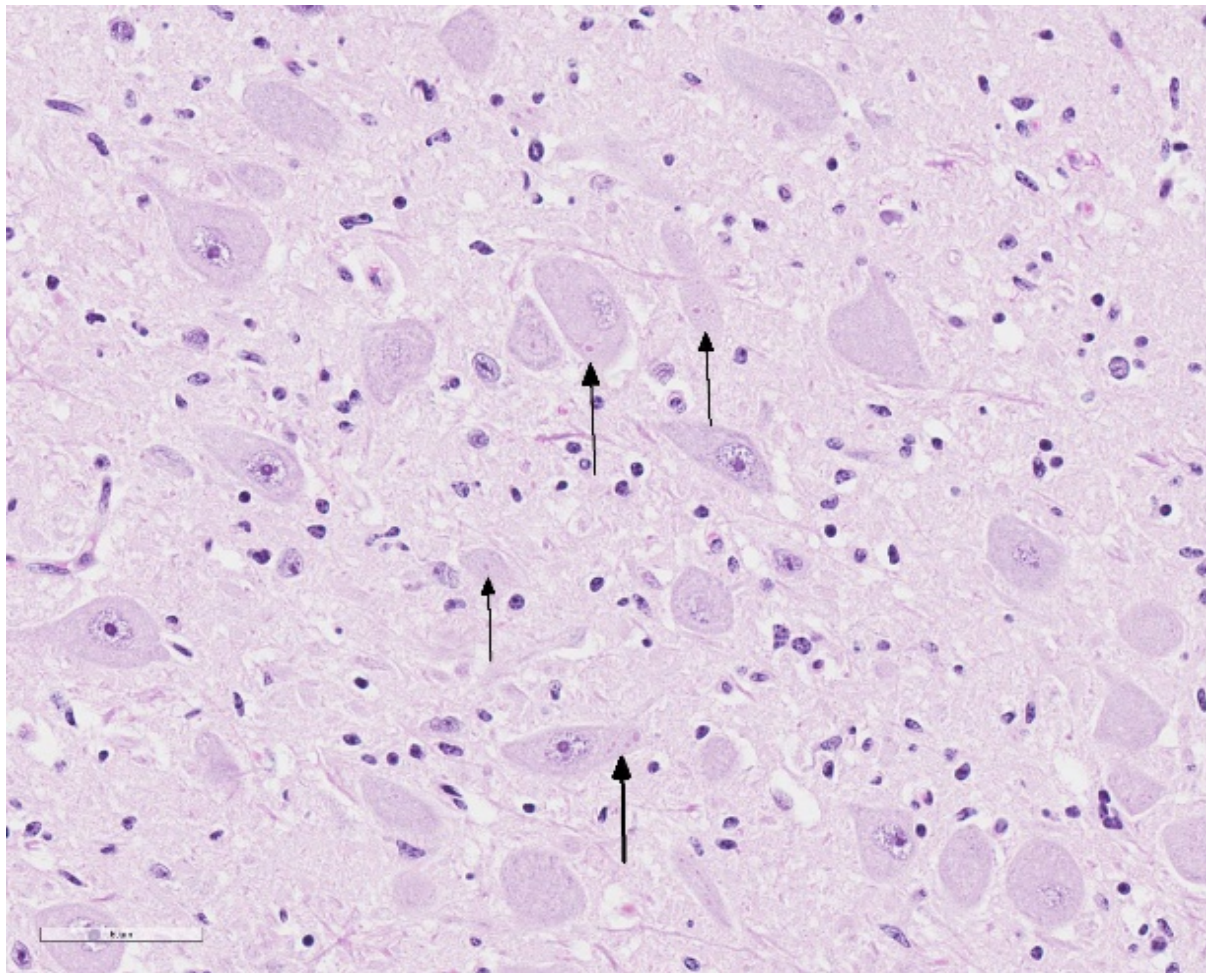
The Middle Ages showed an advance in the diagnosis of clinical rabies with hydrophobia described in affected humans (and noted to be absent in dogs), paralytic rabies, and the need for thorough cleansing of bite wounds as a potential treatment (although the agent was still considered to be a toxin). Religion impacted early vaccination, with dogs vaccinated with a white-hot “Key of Saint Hubert” to prevent

the contraction of rabies, and eventually was applied to the wounds of people bitten by dogs believed to be rabid. Alternatively, an incision would be made in the unfortunate bite victim, and threads from the Saint's cassock were implanted, accompanied by prayers and fasting. Patients not responding to these surefire cures were unfortunately suppressed between mattresses (an alternative form of treatment in Europe until the nineteenth century).⁷

The Renaissance saw rabies as a major entry in medical texts of the time, with an entire volume written on it by Dr. Julien Le Paulmier. The practices at Saint-Hubert

were condemned, and cleansing the wound thoroughly was considered a beneficial practice among clinicians. As the saliva of infected dogs was now being looked at as the potential vector for transmission of the disease, an enterprising Prussian artillery general Kazimierz Siemienowicz tried filling hollow artillery shells with the saliva of rabid dogs and firing them at the enemy in the mid 1600's.⁷

The exploration of the new world brought dog-associated rabies to a continent which had largely recognized only bat-associated rabies, and spread to local wildlife such as mongooses in the Caribbean islands.⁷



Brainstem, 5-month-old calf. Within the subependymal brainstem nuclei, neurons often contain one or more irregularly round 2-3µm intracytoplasmic inclusions (Negri bodies) (HE, 400X).

In Renaissance Europe, the perception of the pathogenesis was evolving. The current thought was that rabies was transmitted by “seeds” (*semina*) in the saliva, and that transmission after a bite wound was an inconstant event. The first 15-day quarantine period was proposed by Joseph-Ignace Guillotin (yes, that Guillotin) in 1776. Conversely, he also suggested inoculation experiments on prisoners awaiting capital punishment (which was supported by Louis Pasteur years later), but thankfully this practice did not occur. Advances in treatment continued to lag behind, with application of the “hair of the dog” to the wound, and even eating omelets flavored with “dog-rose root” (previously suggested over a fifteen hundred years before) as examples of treatments of the time.⁷

In the early 1800’s, the newly minted “scientific approach” made great strides on elucidating the transmission of rabies and potential prevention. In 1813-1814, several researchers in Europe clearly transmitted the disease with the saliva of rabid dogs humans, and sciatic nerve tissue from a rabid cat. Francois Magendie, 30 years later, first suggested the agent was not a poison but a virus, and in 1879, Paul-Henri Duboue first proposed the theory of neuronal transmission.⁷

It was in this time of significant advance that Louis Pasteur was developing a live attenuated vaccine developed by a dessication technique and successfully used on dogs subsequently challenge with live rabies virus. The first human trial, tried on a child who was exhibiting sign of rabies, was met with failure, but a second trial, on 9-year-old Joseph Meister, only two days after the bite of a rabid dog and before the demonstration of any clinical signs was a success. Over 11 days, a total of 13 injections with initially attenuated and progressively more virulent virus allowed

Master Meister to develop immunity against the virus and survive. A second successful attempt three months later in another child proved the efficacy of post-exposure prophylaxis, and since that time, PEP has saved countless lives around the world. In 1932, the League of Nations published records of over 115,000 patients that had received PEP, with a combined rabies mortality of only 0.4%.⁷

Research on the virus itself proceeds apace during this time. In 1903, Adelphi Negri and Lina Luzzani-Negri described the first RABV-neuronal interaction and the cytoplasmic inclusions that still bear their names. The virus itself was first visualized ultrastructurally in the early 1960s, and its viral genome was mapped in 1988. These and many other advances allowed modification and shortening of PEP protocols, and laid the groundwork for marked advances in vaccine research.⁷

However, these advances have only transformed rabies from a globally prevalent scourge, to a global neglected disease, following its eradication in North America and Europe. As of 2017, an estimated 59,000 people die each year of rabies, easily eclipsing similar deaths from Ebola outbreaks. Current attempts to curb these numbers include significant vaccination efforts in animal species, and human rabies vaccination in endemic areas.⁷

There was spirited discussion among participants on formulating a morphologic diagnosis for the relatively minimal inflammation in this case. The term meningitis was ultimately used (although no inflammation was seen in the meninges proper, as the lymphocytes and plasma cells appeared to be confined with Virchow-Robin’s space around vessels, (an extension of the meninges). Other participants

preferred “perivascular encephalitis” but the inflammation did not appear to extend into the adjacent brainstem parenchyma, and still others preferred “perivascular cuffing.”

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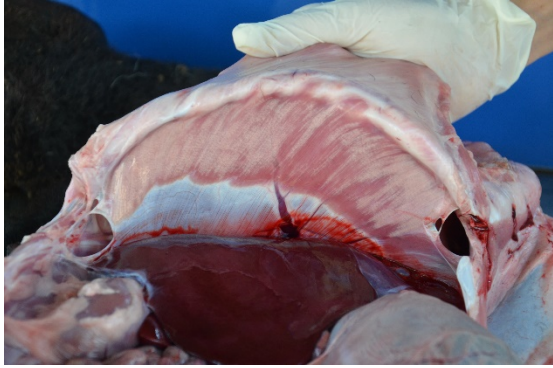
CASE III: 19N153 (JPC 4134352).

Signalment: 3-month-old, male, mixed breed, *Ovis aries*, sheep.

History: In a property with 108 ewes with their lambs with average age of 3 months, 13 lambs got sick. Signals were first noticed by the animals’ caretaker on April 4, 2019, and included paresis, decubitus, nasal discharge, and cough. On April 6, 2019, 10 affected lambs had died, two of which were submitted to necropsy. The remaining three were referred to the Veterinary Teaching Hospital (VTH). Clinical examination showed rales on pulmonary auscultation, hyperemic ocular mucous membranes, and mucous nasal discharge, and the lambs were treated with Se/Vit E, flunixin meglumine and Ceftiofur. Two of them completely recovered and one died on the second day from the start of treatment. The results of the bloodwork performed at the VTH are presented in Table



Hindlimb, lamb. The heavy muscle groups of the hind limb are partially white and opaque, with a chalk-like appearance. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, <https://www.ufms.br/>)



Diaphragm, lamb. The diaphragm has white and opaque stripes as if they were painted-brushed. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, <https://www.ufms.br/>)

1. Some of the lambs that remained at the property presented a subclinical increase in the muscle enzymes activity. In total, three lambs were necropsied, and presented similar gross and histological lesions.

Lambs were fed with 33 kg of ration manufactured on the property that included soybean hull, soybean meal, calcium carbonate and 63 kg of silage from Mombasa grass. Mombasa silage was being offered for the last 48 days. Previously sorghum silage has been provided to the lambs. The lambs were vaccinated and dewormed at 30, 60 and 90 days. The ewes were fed the ration at night and spent the day in Tifton pickets.

Gross Pathology: The cadaver is of an intact male lamb, undefined breed, with a brown wool coat with light areas, in a fair body nutritional status. The conjunctiva is of normal color, and the oral mucosa is pale. There is opacity of the left cornea (probably due to trauma while in decubitus). Multiple, focal, small to extensive, white-opaque chalk-like areas are observed in several muscle groups (Figure 1). The intensity of the lesions varied from mild (+) to severe (+++).

These lesions distributed bilaterally and symmetrically in following muscles: supraspinous (++), radial carpi (+), lateral digital extensors of the carpus, infra-spinosus (+), triceps (+++), brachiocephalic (++), gluteus medius (+++), semitendinosus (++), semimembranosus (+++), vastus medialis, (+++), gastrocnemius (+++), vastus lateralis (++), longissimus dorsi (+++), diaphragm (++) (Figure 2), and tongue (+) and muscoli colli.

The craniodorsal region of the right (the side of decubitus) lung is diffusely dark-red, firm and without crepitation. This aspect extends to portions the right caudal lobe. Large quantities of *Haemonchus contortus* were in the abomasal lumen, admixed with pasty dark-red abomasal content.

Microscopic Description: Skeletal muscle (semimembranosus): Transverse and longitudinal sections of the skeletal muscles were analyzed. Multiple myofibers are either reduced in size or markedly swollen. Segments of affected myofibers are homogeneously hypereosinophilic, with loss of sarcoplasmic striations and, lumpy cleavage of many myofibers (floccular degeneration); the nucleus of these myofibers is pyknotic and shrunken; these changes are more evident in cross-sections. The sarcoplasm of the affected myofibers is moderate to markedly cluttered by fine to dense, basophilic, and birefringent granular material (mineralization). Randomly, in the endomysium, small foci of lymphocytes, plasma cells, and macrophages are seen. Also, satellite there is marked proliferation and swollen of satellite cells, and occasional multinucleated cells and nuclei with mitotic

figures (attempts in regeneration) can be seen in the cytoplasm of degenerating/necrotizing myofibers. Some myofibers are slender, tapered and clear and contain multiple satellite cells arranged in rows in the sarcoplasm (myotubules). This latter finding is most often observed in longitudinal sections.

Within the lumen of pulmonary alveoli and bronchioles are small or moderate amounts of inflammatory cells, predominantly intact neutrophils. In the lumen of the alveoli, there is also amorphous eosinophilic material (edema), eosinophilic fibrillary material (fibrin) and moderate quantities of alveolar macrophages. Occasionally, in the bronchial lumen, small, long, slender fibers measuring about 100 μm in length and the slightly birefringent wall and light center are seen. Those structures are compatible with plant fibers. There are sparse hemorrhagic foci.

There is moderate hepatocellular vacuolar degeneration. There are no lesions in the spleen, kidney, adrenal, myocardium, intercostal, diaphragm, and encephalon.

Contributor's Morphologic Diagnosis:

- 1) Necrotizing acute or subacute myopathy, multifocal, polyphasic, with prominent calcification and myofiber regeneration
- 2) Lung, bronchopneumonia, suppurative, multifocal, moderate, associated with scattered intrabronchiolar vegetable fibers (consistent with aspiration pneumonia) (not submitted)

Contributor's Comment Nutritional myopathies are important diseases of

calves,^{1,18} lambs, swine,¹⁹ and foals,¹⁵ but has been described occasionally in more than 20 animals species including rabbits,¹³ birds,^{11,19} goats,¹⁷ reptiles,¹⁰ and man.¹⁹ The condition was first described in the 1930s and has since been attributed to Se/Vit E deficiency.¹³

The disease is a dominant part of the veterinary literature on muscle diseases⁸ and is also known as nutritional myodegeneration, white muscle disease, stiff-lamb disease, nutritional muscular myopathy (although use of this latter denomination is not recommended). Several syndromes are associated with deficiency of Se, Vit E, or both in domestic animals, such as nutritional myopathy, degenerative myelopathy in horses, degenerative encephalopathy in birds, retention of placenta in cattle, hepatitis dietetica in pigs, mulberry heart disease in pigs, exudative diathesis in pigs⁷ and chickens, steatitis (yellow fat disease) in cats, and immune system compromise;¹⁹ in people this nutritional deficiency is known in China for inducing a degenerative cardiomyopathy known as Keshan disease.¹⁹

The pathogenesis of nutritional myopathy due to Se/Vit E deficiency is not entirely understood. In these cases, as is customary, the usual suspects are lined up, and free radicals (FRs) are the main ones. Se/Vit E are responsible for the protection of cell membranes against the action of FRs, which mediate cell membrane damage.¹⁶ FRs are chemical specimens that have an unpaired electron in the most external orbit of the atom. FRs include reactive oxygen species such as superoxide (O_2^-) and reactive nitrogen species such as NO .¹⁶ Because they

possess this unpaired electron, FRs readily reacts with organic substances causing the peroxidation of the phospholipids, proteins and nucleic acids of the cellular membrane, leading the cell to necrosis. The protection of

membranes is partly the responsibility of selenium-containing enzymes, and more than 30 selenoproteins are known⁷ and are part of the glutathione peroxidase/glutathione reductase system.^{4,9}

Test	♀1	♀2	♂3	Reference value
RBC's 10 ⁶ /μL	10,89	13,59	7,3	9-15
Hemoglobin (g/dL)	8,2	9,7	5,6	9-15
PCV (%)	28,8	35,6	20,2	27-45
MCV (fL)	26,4	26,2	27,7	28-40
MCH (g/dL)	28,5	27,2	27,7	31-34
Leukocytes (mm ³)	25.500	14.600	7.600	4.000-12.000
Neutrophils (mm ³)	20.910	9.928	4.636	3.000-6.000
Lymphocytes (mm ³)	4.590	4676		1.000-9.000
Platelets (mm ³)	1.574.000	1.337.000	1.406.000	300.000-600.000
AST (UI/L)	1.839,10	8.268,40	14.174,50	98-278
Creatinine (mg/dL)	0,4	0,3	0,4	1,2-1,9
Creatine kinase (UI/L)	2.068,10	14.333,70	57.384,50	8,1-12,9
GGT (UI/L)	-	-	-	20 a 52
BUN (mg/dL)	-	-	-	17,12 a 42,8
Tortal protein (g/dL)	5,93	5,46	5	6,0 a 7,9
Globulin (g/dL)	3,03	2,96	2,8	3,5 a 5,7
Albumin (g/dL)	2,9	2,5	2,2	2,4 a 3,0

Table 1. Nutritional myopathy. Laboratory tests performed in the three affected lambs referred to the VTH. Lamb # 3 died. The other two recovered after treatment.

If any condition deprives the organism from these mechanisms, the cellular membranes

become physiologically defective, allowing the influx of calcium into the cytosol,

resulting in the accumulation of calcium in the mitochondria. Damaged mitochondria fail to maintain the energetic needs of the cell, which results in cell necrosis, which manifests, in the myofiber, as floccular necrosis. The membrane injury allows the leakage of enzymes such as creatine kinase (CK), whose increased activity into the serum is a sign of muscle injury.⁷ One way to confirm the disease is by determining low levels of selenium in the food and carcasses of the affected animal, increased serum CK activity and histological findings of muscle degeneration.⁷

Several factors influence the transfer of Se from the soil to the plants, among them soil alkalinity (which favors the absorption of Se), type of plant (plants differ in their capacity to store selenium) and the presence of sulfur that competes with selenium, which is lower in the spring and in the rainy season.^{7,8}

Data on selenium levels in Brazilian soils are scarce. However, outbreaks of nutritional myopathy are described in the South,² the Northeast,¹ and North³ of the country. In the South outbreaks occur mainly from July to August (winter, rainy season). Morbidity may be low but may reach 20%.

Feed with concentrates with a high content of fatty acids favors the occurrence of Se/Vit E deficiency. On the other hand, in animals fed concentrate, the availability of selenium may also be decreased as a consequence of the low pH of the rumen. Animals may die acutely without premonitory signs or after onset of apathy, dyspnea, and blood-filled foamy nasal discharge. There is marked tachycardia

(150-200 bpm) and temperature is normal.⁷ In the acute form the treatment is usually ineffective. The most common form is the subacute with clinical course from a few days to a week, and mainly affects calves and lambs. The affected animals can be found in decubitus.

Clinical signs include muscle stiffness, difficulty in locomotion, muscle tremors, abnormal postures, apathy, and death. Occasionally it is possible to observe bilateral and symmetrical swelling of the gluteus, dorsolumbar muscles and muscles of the shoulders. The involvement of the diaphragm and the pharyngeal and esophageal muscles is responsible for the dysphagia seen in clinic cases.⁷ Dysphagia results in aspiration pneumonia, so typical in cases of nutritional myopathy² and that occurred in the lambs of this outbreak. The subacute form (as in this report) generally responds well to treatment with Se/Vit E and animals recover within 3-5 days. Myoglobinuria is not a common sign in young animals but may occur sporadically in adult animals.⁸

Gross lesions are seen in the skeletal muscles and myocardium. They are bilaterally symmetrical and affected the muscles with a heavy load. The type of affected muscle varies with the age of the affected animal. In lambs the muscles of the neck and tongue (involved in suckling) are the most affected. In slightly older animals, muscles of the shoulder, thigh and diaphragm are most affected.⁸ Calcification of well-developed lesions leaves the chalky (opaque white) muscle, which gives rise to the name and "white muscle disease."²

Microscopic changes in the myofibers vary somewhat between species, but are mostly the same. Usually they are polyphasic and include hyaline degeneration with loss of myofiber striations, floccular necrosis,

marked proliferation of satellite cells, and an influx of neutrophils and macrophages into the sarcoplasmic tube. Secondary

Name of Condition	Epidemiology and Pathology
<i>Senna occidentalis</i> and <i>S. obtusifolia</i> toxicosis	Degenerative lesions are marked in the skeletal muscles and very mild, if any, in the myocardium. Mineralization of muscle lesions are mild or absent. Mostly monophasic lesions. Myoglobinuria is a common clinical sign. Treatment with Se/vit E is ineffective. ⁵
Ionophore toxicosis	Ionophores are food additives, therefore the disease occurs in animals being fed commercial rations. Lesions are in both, myocardium and skeletal muscle. In ruminants, lesions are prominent in the myocardium. Mineralization of the lesions is not a feature. Mostly monophasic lesions. Myoglobinuria is part of the clinical picture. Treatment with Se/vit E is ineffective.
Nutritional myopathy (Se/vit E responsive disease)	Usually affects fast-growing young animals (2-4 month-old). Degenerative lesions occur in both skeletal and myocardial muscles. Myocardial lesions are not present in all cases. ⁷ There is extensive mineralization of the lesions, which gives an opaque soft chalk-like gross appearance to the lesions (which does not occur in the other degenerative diseases mentioned in this table). Mostly polyphasic lesions. When instituted early in the course of disease treatment with Se/Vit E is effective. Myoglobinuria is usually not part of the clinical picture, except in sporadic cases in adult animals.
Gossypol toxicosis	Monogastric animals, such as pigs, are more susceptible to gossypol toxicity than ruminants. Necropsy lesions do not always include muscle degeneration (Mostly polyphasic lesions). Acute hydrothorax, hydropericardium, ascites and subcutaneous edema are part of necropsy findings. Hepatic lesions of centrilobular necrosis in pigs intoxicated with gossypol can be severe.
Downer syndrome	Usually affects well-nourished, well-fed, animals with large muscle masses. The lesion is well demarcated and focal or focally extensive and results from pressure (ischemia). Lesions tend to be polyphasic. It affects the parts in decubitus, usually those close to bony prominences. Muscle hemorrhages, ulcerative lesions on the skin that correspond to the decubitus site may be associated with these lesions and aid in the differential diagnosis.

calcification is marked. The regeneration of muscle fibers is remarkable.¹³

The diagnosis is based on the characteristic clinical signs, associated to the clinical pathology and anatomopathological findings. Segmental necrosis of myofibers with calcification is typical of this disease, but not diagnostic. Confirmation of the diagnosis requires the determination of levels of selenium and α -tocopherol in tissues (renal cortex and liver for Se and liver for α -tocopherol). As the serum activity of glutathione peroxidase is highly correlated with blood levels of Se, the activity of this enzyme in the blood is used to assess levels of Se in tissues. Se and α -tocopherol analysis are useful because they differentiate cases of segmental myonecrosis from causes other than Se/Vit E deficiency.

The differential diagnosis should include toxic myopathies such as those caused by *Senna occidentalis* (coffee senna)⁵ and *S. obtusifolia*⁶ that frequently produce intoxication in cattle in this part the world, or by ionophore antibiotics, another common livestock toxicosis in this region. In cases of toxic myopathy, the agent must be investigated in the feed and the presence of the plant should be determined in the pasture. Some aspects of differential diagnosis are shown in Table 2.

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JPC Diagnosis: Skeletal muscle: Myocyte degeneration and necrosis, polyphasic, diffuse, moderate, with mineralization and rare myocyte regeneration.

JPC Comment: Another excellent and far-ranging discourse on the entity of nutritional myopathy by the contributor allows us to explore some less common avenues of Vitamin E deficiency, this time the human disease associated with hypovitaminosis E.

While there are eight different forms of Vitamin E, four each in the tocopherols and four in the tocotrienols (which all are absorbed in the small intestine), the liver can metabolize only one, alpha-tocopherol. The other seven forms are excreted. Low Vitamin E diets are seen in emerging countries; in developing countries, diseases impacting absorption are the most common cause. In the US, although studies have shown that 90% of men and 96% of women 19 years or older have diets deficient in Vitam E, only 0.1% of this demographic have deficient serum levels of alpha tocopherol.

The most common causes of Vitamin E deficiency in humans include premature low birth weight (premature infants have low vitamin E reserves due to limited placental transfer), mutations in hepatic tocopherol transfer proteins, various causes of fat malabsorption, cystic fibrosis (as patients are unable to secrete pancreatic enzymes which help absorb within E, short bowel or inflammatory syndromes, abetalipoproteinemia, and a genetic mutation of chromosome arm 8q.

The known beneficial effects of Vitam E in humans are in the areas of antioxidants,

immunomodulatory, and antiplatelet effects. Vitamin E supplementations may impact the development of coronary artery disease by preventing oxidative changes to LDLs. It enhances lymphocyte proliferation while decreased the production of prostaglandin E2 and serum lipid peroxides. By the combination of decreasing LDL oxidation and reducing PGE2, this in turn reduces platelet aggregation as well as inhibiting protein kinase C.

True disease is uncommon in hypovitaminosis E in humans. One of the manifestations arises from a lack of its antioxidant properties in the nervous system. One particular genetic disease arising from this deficiency is known as ataxia with isolated vitamin E deficiency (AVED). This autosomal recessive disorder results from a mutation in the gene which codes for the alpha-tocopherol transfer protein and maps to chromosome 8q13. It manifests from the age 2 onward with ataxia, hyporeflexia, and difficulty with upward gaze. The deficient alpha-tocopherol transfer protein delivers Vitamin E to Purkinje cells and other neurons, leading to free radical stress, oxidative damage and cellular dysfunction in these cells. A similar condition, Friedreich ataxia, may mimic AVED, but results from a genetic mutation of the FXN gene, which results in oxidative stress due to iron buildup in neuronal mitochondria.

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CASE IV: AFIP 2018 053/18 (JPC 4119040).

Signalment: 5 year, 8-month-old spayed female DSH cat (*Felis silvestris*)

History: This cat presented with polyuria, polydipsia and cystitis. A diagnosis of diabetes mellitus was confirmed. The cat



Pancreas, cat. There is mild pancreatomegaly and several lightly colored nodular masses scattered though the parenchyma. (Photo courtesy of: Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Section of Pathology, BOX 7028, SE 750 07, Uppsala, Sweden, <https://www.slu.se/en/departments/biomedical-sciences-veterinary-public-health/>)

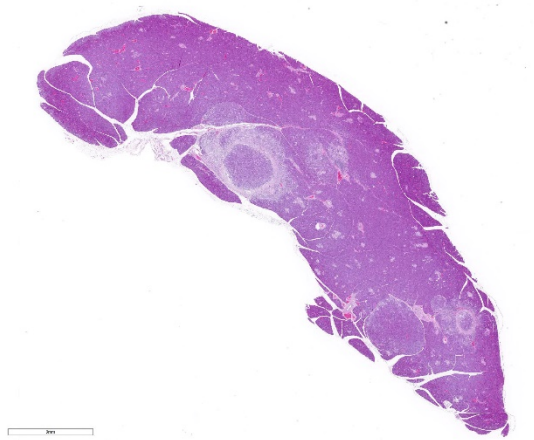
was treated with insulin, but blood glucose concentrations remained high despite treatment. For this reason, a presumptive diagnosis of insulin resistant diabetes was made, and the concentration of insulin-like growth factor (IGF)-1 in serum was measured. Later, the cat developed severe and recurrent vomiting, whereby it was humanely euthanized and submitted for necropsy.

Gross Pathology: The cat was in good body condition with well-developed musculature. The pancreas was moderately enlarged and showed multiple lightly colored, homogenous, nodular structures measuring up to 5 mm in diameter. The pituitary gland was moderately enlarged.

Laboratory results: S-Glucose >20 mmol/l (reference range 3.5-6 mmol/l)

S-Fructosamine >500 $\mu\text{mol/l}$ (reference range 190-350 $\mu\text{mol/l}$)

Serum Insulin-like Growth Factor (S-IGF-1) >3050 $\mu\text{g/l}$ (reference range 100-1200 $\mu\text{g/l}$)



Pancreas, cat. A section of pancreas is submitted for examination. There are numerous nodules throughout the section, some with prominent fibrous capsules, and adjacent areas of pallor with loss of exocrine tissue. (HE, 7X)

Histologically, a well-circumscribed proliferation of well-differentiated acidophil cells was confirmed in the pituitary gland, consistent with an acidophil cell adenoma.

Microscopic Description: The exocrine parenchyma shows several well-circumscribed nodules, composed of variable amounts of well-differentiated acinar and ductal cells, often with reduced cytoplasmic staining properties. Surrounding nodules, and multifocally separating lobules as well as acinar and ductular structures, are variable amounts of fibrous tissue, multifocally expanded by numerous lymphocytes and small numbers of plasma cells. Multifocally, ductular structures are dilated and show epithelial attenuation and loss. Within lumina are moderate numbers of neutrophils and macrophages, as well as smaller amounts of eosinophilic, amorphous material (secretion), occasionally admixed with cellular debris. Diffusely, Langerhans's islets show moderate to marked cellular loss and are expanded by abundant amphophilic to lightly eosinophilic extracellular amorphous material (amyloid). Multifocally, small numbers of cells within the islets show an expanded vacuolated cytoplasm.

In Congo-red stained section (not provided), the extracellular material in the islets was confirmed to be amyloid by exhibiting apple-green birefringence in polarized light.

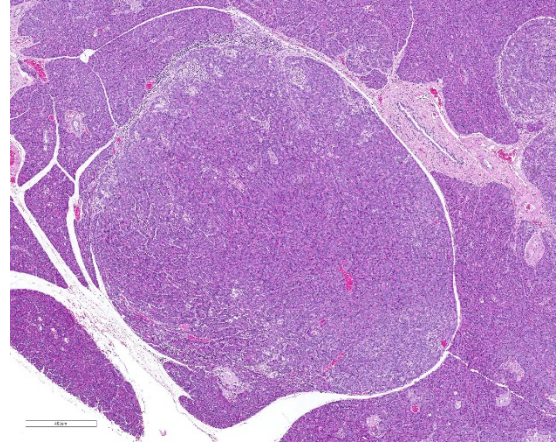
Contributor's Morphologic Diagnosis:

1. Pancreas, multifocal nodular hyperplasia/adenomas
2. Pancreas, pancreatitis, multifocal, interstitial, chronic, moderate, lymphocytic with multifocal acinar/ductular dilation and neutrophilic inflammation
3. Pancreas: islet amyloidosis, diffuse, severe, with cellular degeneration and loss

Contributor's Comment Diabetes mellitus is a disease characterized by loss of glycemic control with resulting fasting hyperglycemia. In cats, insulin resistant diabetes mellitus, resulting from inadequate tissue response to high insulin level is frequent, similar to diabetes mellitus type II in humans.¹⁰

Several pancreatic changes of the endocrine islets are associated with diabetes mellitus in cats, including loss of insulin-producing β -cells and amyloidosis of the islets.

According to a recently published article, diabetic cats had approximately 65% lower median number of insulin-producing cells compared to control cats.¹⁸ The mechanism of amyloidosis is related to co-secretion of insulin and islet amyloid polypeptide (IAPP).¹¹ The presence of amyloid can be verified histologically by staining with Congo Red, as was performed in this case, with amyloid protein exhibiting apple-green birefringence in polarized light.⁴ Thioflavin-S or T can also be used to detect amyloid, and may be useful due to poor staining with Congo red in cats.^{4,8} Historically, amyloidosis has been considered a consistent feature of diabetes mellitus in



Pancreas, cat. There are several large areas of exocrine hyperplasia scattered throughout the section. (HE, 58X)

cats, and sometimes the cause of insulin dysregulation.^{9,16} However, this association and causality has been questioned in recent years, and a newly published paper failed to find increased deposits of amyloid in the pancreas of diabetic cats compared to controls.¹⁸

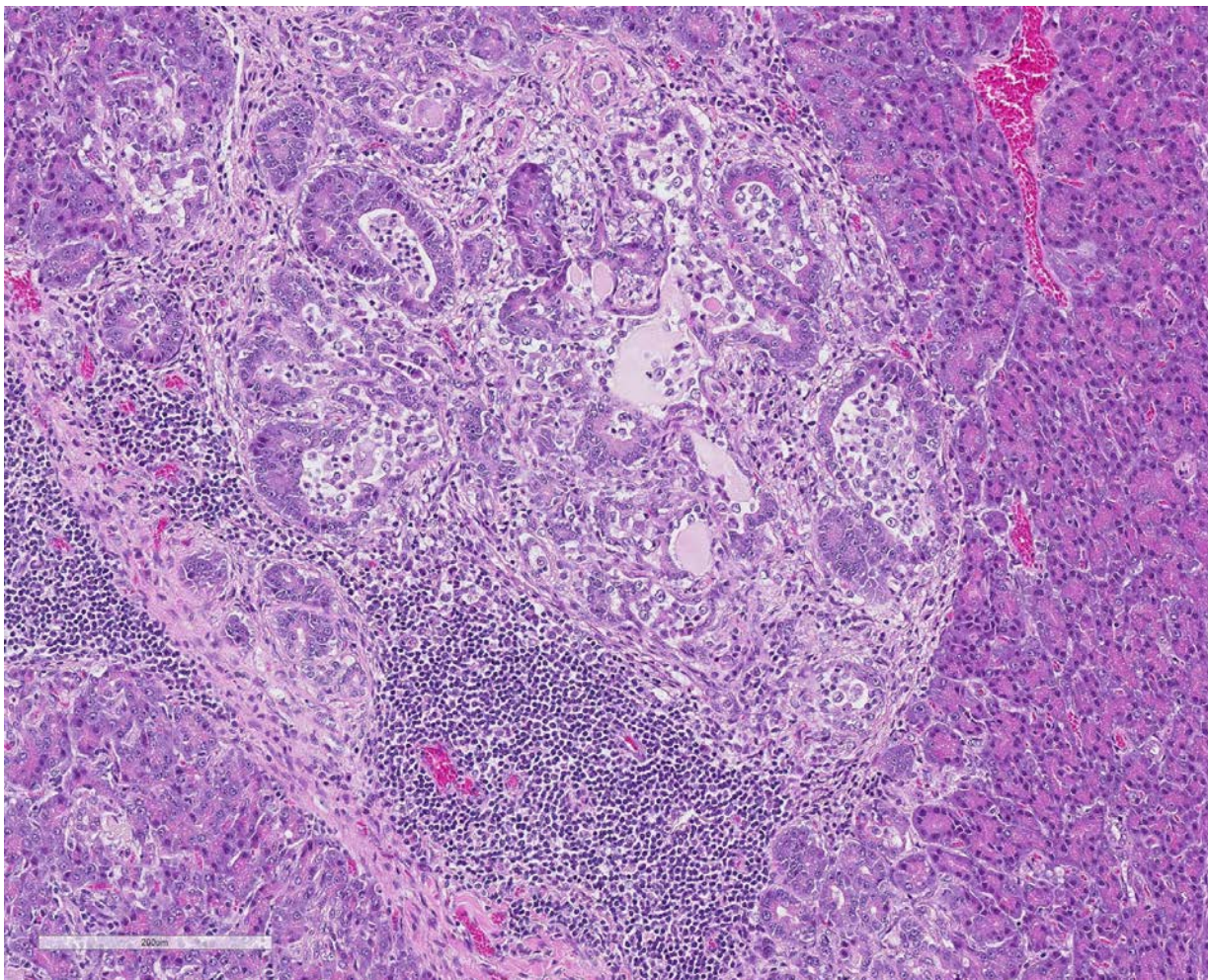
The degree to which inflammation plays a role in feline diabetes remains controversial. According to one study, the number of neutrophils and macrophages in pancreatic islets was similar between cats with diabetes mellitus and control cats, however the presence of T- and B-cells combined seemed more frequent in islets of diabetic cats compared to non-diabetic cats.¹⁸ In another study, expression of mediators regulating inflammation and oxidative stress was seen in cats with hyperglycemia and obesity, suggesting that these markers increase in the early development of diabetes mellitus.⁸

In addition to diabetes mellitus resulting from inadequate tissue response to high insulin level, a known cause of feline diabetes mellitus is acromegaly.¹⁶

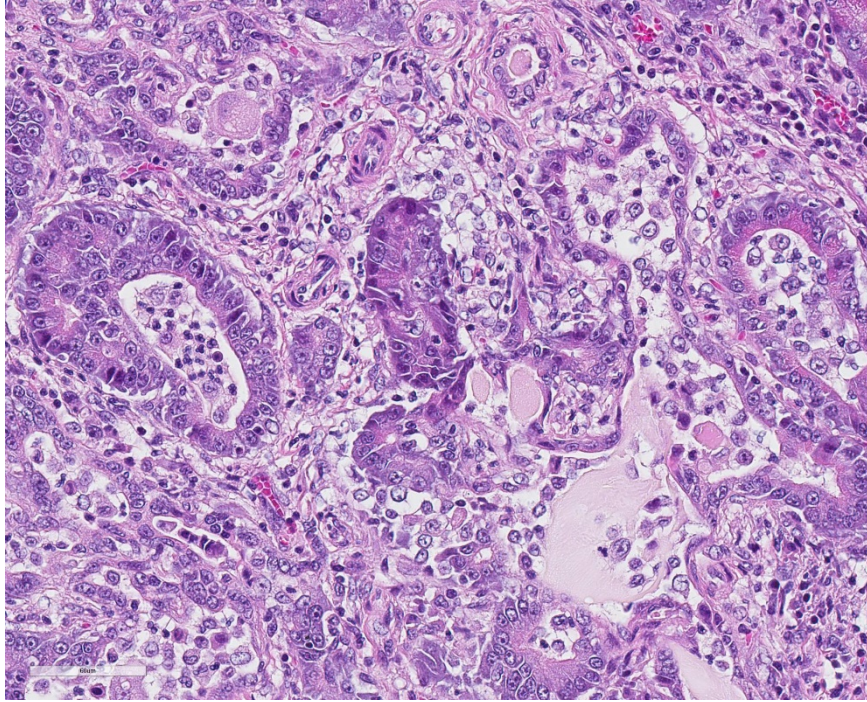
Acromegaly is a clinical condition characterized by overgrowth of connective tissue and enlargement of viscera, resulting from chronic excessive secretion of growth hormone (GH), also known as hypersomatotropism.^{16,19} According to one author, nodular pancreatic hyperplasia is commonly encountered in acromegalic cats on post mortem examinations.¹⁴ Acromegaly may cause diabetes mellitus due to a GH-induced postreceptor defect in insulin action in target tissues.¹⁴ GH measurements in blood has traditionally been used to diagnose acromegaly. However, S-IGF1 has replaced this parameter in recent years, due

to limited GH assay availability.^{3,13} Presence of an acidophil cell adenoma in the pituitary gland, together with severely elevated serum IGF values, suggest that the diabetes mellitus in this case was a result of hypersomatotropism due to neoplastic GH-secretion.

Pancreatitis is frequently diagnosed in cats,; the chronic form being more prevalent than the acute form.² In the acute form, neutrophilic infiltrates along with acinar cell and peripancreatic fat necrosis is observed, meanwhile chronic pancreatitis is dominated by non-suppurative infiltrates of



Pancreas, cat. There are several large areas of exocrine hyperplasia scattered throughout the section. (HE, 58X)



Pancreas, cat. Higher magnification of a focus of interstitial pancreatitis. In these areas, it is difficult to distinguish degenerating from regenerating acini or pancreatic ducts. (HE, 400X)

lymphocytes, accompanied by fibrosis and acinar atrophy.^{6,10} Pancreatitis in cats may coexist with inflammatory bowel disease and cholangitis.¹ Moreover, pancreatitis is often observed in conjunction with a diagnosis of diabetes mellitus, and may be subclinical.^{5,18} Anecdotally, the concurrent condition of diabetes mellitus and pancreatitis can make glycemic control more “brittle”.⁵ However, it is not clear whether the pancreatitis leads to diabetes mellitus, or if the diabetes mellitus causes pancreatitis.⁵ It has been shown that the number of neutrophils is increased in pancreatic exocrine tissue in cats with hyperglycemia.¹⁸ According to another study, the degree of inflammation of the exocrine pancreas is not significantly associated with diabetes nor ketoacidosis. However, the mitotic index of acinar cells, as measured by Ki67 and

PCNA, is increased in cells in the near vicinity of pancreatic islets in cats with diabetes, possibly due to chronic pancreatitis.¹⁷

The gross nodular changes in the pancreas corresponded histologically to well-defined benign proliferations of exocrine pancreatic tissue. In summary, these were determined to be representative of nodular hyperplasias or adenomas. Nodular hyperplasia can be seen in several organ systems of aging individuals including the

liver, adrenals, thyroid glands, spleen and pancreas.^{10,13,17} In the pancreas these changes are usually considered clinically silent. In other organs, nodular hyperplasia has been discussed as a response to tissue injury or due to chronic pituitary hormone stimulation.¹² In this case, it is unclear if the nodular changes are purely hyperplastic or represent an adenomatous change. Possibly, the chronic inflammation could contribute to the increased amount of fibrous tissue surrounding nodules.

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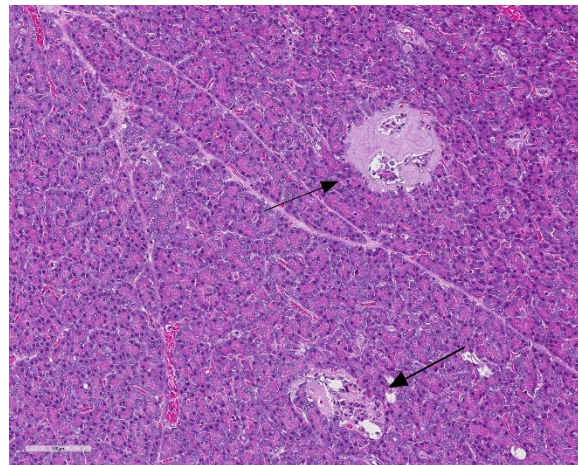
JPC Diagnosis: 1. Pancreas: Pancreatitis, interstitial, necrotizing and lymphohistiocytic, chronic, multifocal mild to moderate.

2. Pancreas, islets of Langerhans: Amyloidosis, diffuse, severe.

3. Pancreas, exocrine tissue: Hyperplasia, nodular multifocal, moderate.

JPC Comment: While the theoretical interplay of the three visualized lesions of chronic interstitial pancreatitis, islet amyloidosis, and exocrine hyperplasia, as well as the fourth lesion of an enlarged pituitary (which was neither submitted or further described in this case) and its discussion by the contributor is interesting to ponder; the participants had seen these three lesions independently and in various combination frequently in older cats, and were reticent to assign a relationship between any of the three, or the concomitant increased levels of serum growth hormone. (Also interesting is that this particular cat was less than 6 years of age.) Amylin, or islet associated peptide is toxic to islet cells in addition to resulting in effacement and atrophy as a result, but the presence of islet amyloid in the cat should not be interpreted as *prima facie* evidence of diabetes in the cat.¹⁰

The contributor mentions the possibility of elevated growth hormone levels and acromegaly in this particular individual as a potential cause of diabetes. Diabetes in acromegalic cats is a result of the catabolic



Pancreas, cat. Islets are largely replaced by amyloid. (HE, 203X)

effects of growth hormone, which causes insulin resistance through the generation of a postreceptor defect in the action of insulin or target cells with resulting diminished carbohydrate utilization as well as gluconeogenesis.⁷ Various studies have demonstrated the concurrent presence of diabetes mellitus in between 20 and 50% of acromegalic human patients. One study of 1221 diabetic cats demonstrated 26.1% to have elevated serum insulin-like growth factor (secreted by the liver) and of this subset, 89% demonstrated pituitary neoplasms.¹⁵

Another effect of growth hormone release by a pituitary somatotroph tumor in an acromegalic patient is due to the anabolic effects of insulin-like growth factors, which results in increased protein synthesis and tissue growth. Affected cats demonstrate a characteristic clinical appearance (colloquially referred to as a “gangster cat” due to increased body weight, prognathia inferior, a broad face, enlarged paws, respiratory stridor, and often cardiac and abdominal organomegaly). While not seen

in all patients, these changes were not documented in the history of this case.⁷

Chronic interstitial pancreatitis is the typical pattern of inflammation of the pancreas in species other than the dog, and in cats, generally arises from an inflammatory process beginning in the ducts.

Histologically, as seen in this case, ducts may contain exudate, may be dilated or stenotic, and are often surrounded by bands of fibrous connective tissue which may communicate with the interlobular stroma. Aggregates of lymphocytes and plasma cells are not uncommon in inflamed areas.¹⁰

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