



WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 15

16 January 2018

CASE I: 16153 E (JPC 4102152).

Signalment: 7 year old, male, rhesus macaque (*Macaca mulatta*)

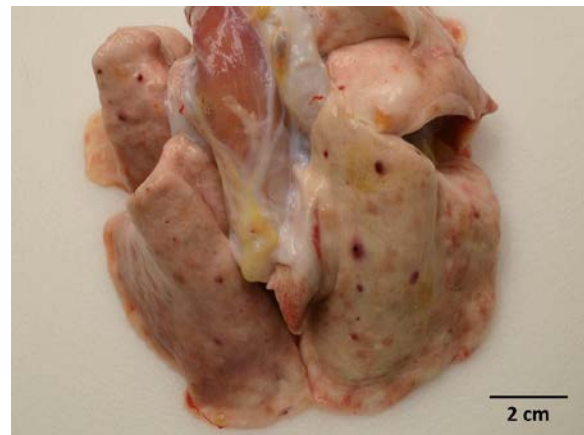
History: This animal received total body irradiation (4 Gy) as part of an experimental protocol, and demonstrated persistent pyrexia (104 °-105 ° F), inappetence, weight loss and was persistently pancytopenic despite aggressive supportive care, blood transfusions and antibiotic therapy (Enrofloxacin, Ceftiofur and Ertepenem). The animal was euthanized 47 days post-irradiation due to poor prognosis and non-response to treatment.

Gross Pathology: Dozens of petechiae, ecchymoses and pinpoint to 1.4 mm diameter, soft, yellow-tan foci and were scattered throughout the parenchyma of both lungs. Additional petechiae and ecchymoses were scattered throughout the left lateral lobe of the liver and urinary bladder. The epicardium of the right ventricle was irregularly thickened by off-white opaque material.

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Laboratory results: Blood culture: No growth
Transmission electron microscopy, epicardial adipose tissue: There were numerous 3-4µm diameter extracellular and intrahistiocytic protozoa with cilia projecting

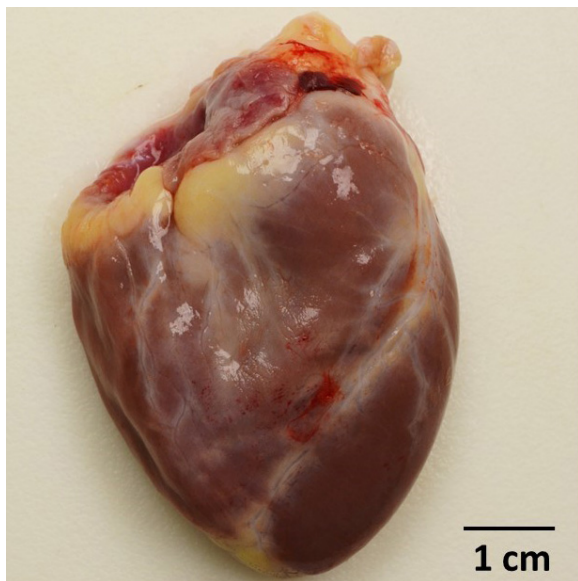


Lungs, rhesus macaque. Ecchymoses, petechiae and soft, yellow-tan foci are scattered throughout the parenchyma of all lung lobes. (Photo courtesy of: Wake Forest School of Medicine Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157 www.wakehealth.edu)

from the lateral margins, often arranged in parallel rows, moderately electron dense grainy cytoplasm, two electron dense nuclei, and a single internal marginated flagellum.

Microscopic Description:

The epicardium and epicardial adipose tissue is infiltrated by coalescing aggregates of lymphocytes, plasma cells, macrophages, and scattered foci of neutrophils, all of which dissect between and separate adipocytes, and extend into the underlying myocardium. Innumerable, free and intrahistiocytic, 3-4 μ m diameter, oval, eosinophilic to amphophilic protozoa are scattered throughout the affected tissue. The protozoa are surrounded by a 10 μ m diameter clear space within the matrix. The protozoa stain positively with Heidenhain iron hematoxylin (HIH); and are negative for periodic acid-Schiff (PAS), Groncott-Gomori methenamine silver stain (GMS), and mucicarmin.



Heart, rhesus macaque. The epicardium of the right ventricle is variably thickened by opaque off-white material. (Photo courtesy of: Wake Forest School of Medicine Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157 www.wakehealth.edu)



Heart, rhesus macaque. The epicardial fat, epicardium, and right atrial and ventricular myocardium contains a cellular infiltrate. (HE, 5X)

The epicardial surface is overlain by sheets of loose, fibrillar eosinophilic material (fibrin), containing scattered lymphocytes and histiocytes.

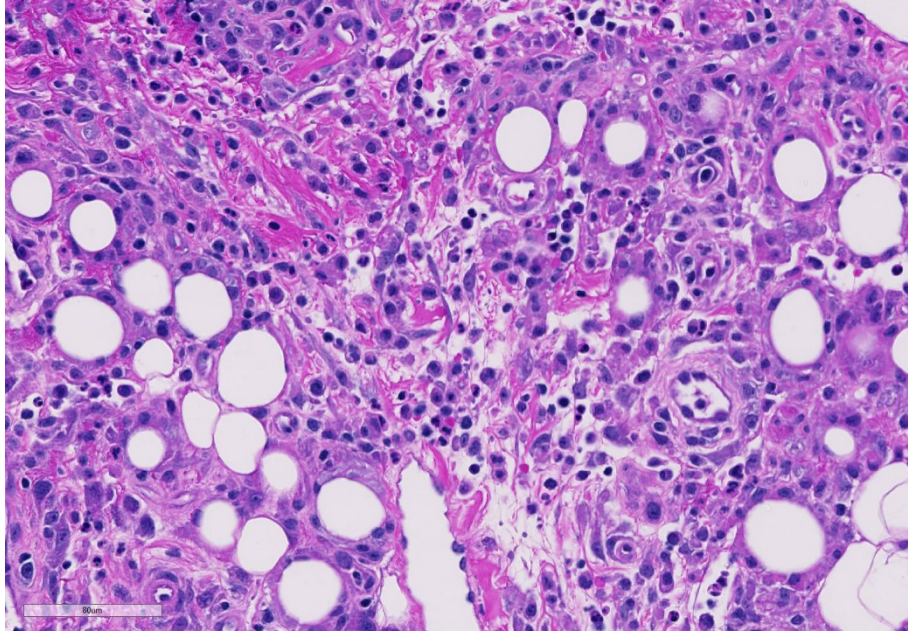
Contributor's Morphologic Diagnoses:

Epicarditis, pericarditis, myocarditis, and epicardial steatitis, regionally extensive, chronic, severe, lymphohistiocytic with protozoa

Contributor's Comment:

Spironucleus species are flagellated diplomonad protozoans which are found as gastrointestinal (GI) commensals and may also act as opportunistic parasites in an array of hosts including birds, fish, and mammals. Generally, the parasites live within the intestinal lumen or crypts, with clinical signs in infected animals either not observed or limited to GI-related symptoms including diarrhea, anorexia, and wasting^{4,11}. Gross pathologic changes depend on the severity of disease, and may include gas-distended intestines, enteritis, and ascites.³

While *Spironucleus* species rarely cause extra-intestinal disease, such incidents have been previously documented in Atlantic salmon¹², Atlantic char¹³, Siamese fighting



Heart, rhesus macaque. Epicardial fibroadipose tissue is infiltrated by moderate numbers of neutrophils, macrophages, lymphocytes, and plasma cells. (HE, 282X)

fish, cichlids¹⁰, and rhesus macaques². Immunocompromised animals have a greater risk of developing an overwhelming infection; for example, athymic mice infected with *Spironucleus* have increased mortality.⁴ Reported pathogenic infections in macaques have been in animals infected with Simian Immunodeficiency virus.^{2,8}

This animal was one of three in a cohort of animals that had received experimental whole body irradiation. All three animals had systemic *Spironucleus* infection where protozoa were positively identified and confirmed by PCR. Speciation is pending. Lesions included polyserositis, epicarditis, ureteritis, cystitis, epididymitis, and pneumonia. One animal had protozoa in the cerebrospinal fluid.

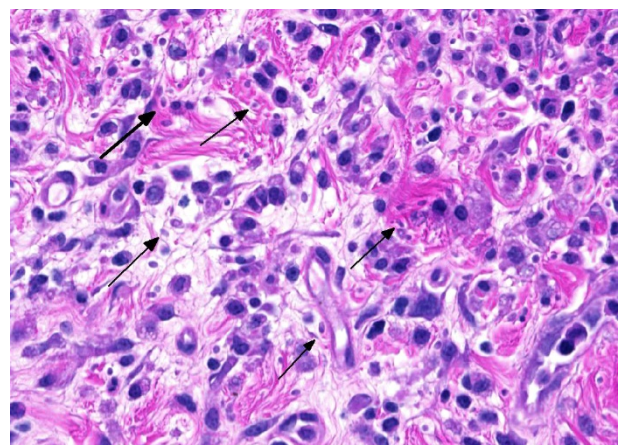
Spironucleus, formerly classed as *Hexamita* or *Octomitus* in some species^{9,11}, may be difficult to detect on routine histologic sections. Histologic stains used to identify them are hematoxylin and eosin (H&E), Giemsa, Gomori's methenamine silver

(GMS), and Heidenhain iron hematoxylin (HIH).¹⁴ The protozoa from the three cases from our institution appeared amphophilic on H&E and Giemsa, were negative for GMS, and prominently stained deep purple with HIH. Other previously utilized techniques for diagnosis include direct smear of intestinal contents, immunochemical techniques on feces, and polymerase chain reaction.^{5,6} Electron microscopy (EM) has also been used as a tool

to positively identify *Spironucleus* species, which, on TEM depending on speciation, range from 2-4x4-20µm have lateral cilia and a recurrent flagellum which arises from an indent in the nuclear membrane near two nuclei, which form an 'S'-shape.^{2,12}

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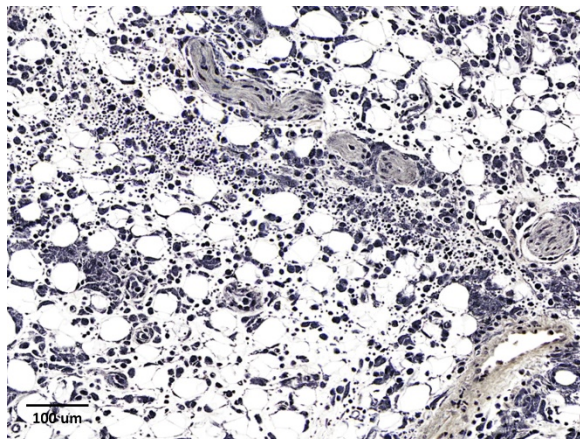
Heart, rhesus macaque. Numerous oval 2-4 extracellular flagellated protozoans are present extracellularly (arrows). Close inspection will also demonstrate one or multiple protozoa within the cytoplasm of macrophages as well. (HE.

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JPC Diagnosis: Heart: Epicarditis and steatitis, histiocytic and neutrophilic, multifocal to coalescing, marked with mild myocarditis and endocarditis and numerous intrahistiocytic and extracellular protozoa.

JPC Comment: The contributor has provided an excellent discussion of this rarely reported systemic condition in immunosuppressed macaques. A very similar case of systemic spironucleosis was included in the 2011-2012 Wednesday Slide Conference (Conference 19, case 3) although the submitted tissue was colon.⁸

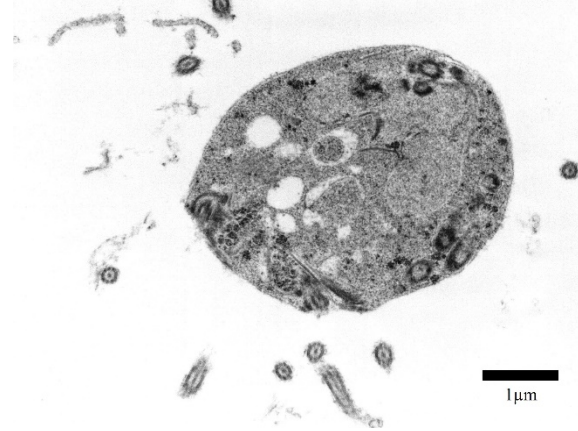
Reports of *Spironucleus* in non-human primates are rare in both health and disease. To date, the rhesus macaque appears to be the only species of non-human primate in which this genus (specifically, *S. pitheci*) has been identified², and all other reports of *Spironucleus* (largely that as a gut commensal) are in laboratory rodent species. Immunosuppression in rodent species may



Heart, rhesus macaque. A Heidenhain iron hematoxylin stain demonstrates the presence of numerous protozoa within the pericardial fat. (Photo courtesy of: Wake Forest School of Medicine Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157 www.wakehealth.edu) (HIH, 200X)

also lead to systemic infection.

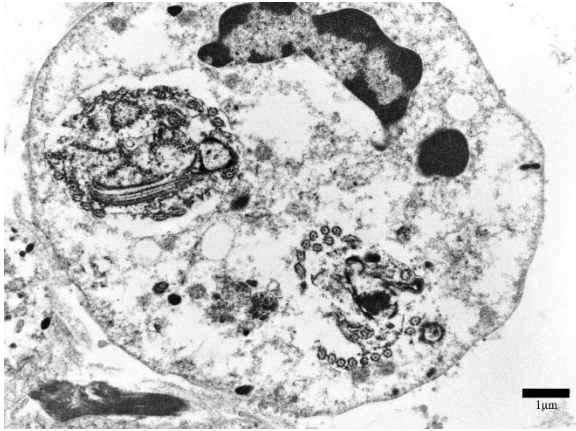
Spironucleus and other related flagellates such as *Giardia* and *Entamoeba* are interesting in their evolutionary development of a highly reductive organelle to take the place of mitochondria, known as a “mitosome” (or in the case of *Spironucleus*, the “hydrogenosome”).¹ These organelles, like mitochondria, have a double membrane, and are associated with a number of proteins closely related to those found in mitochondria. Unlike mitochondria, mitosomes do not contain their own genes; genes for their genesis and function are



Heart, rhesus monkey: Transmission electron microscope (TEM) image of the pericardial fat: There is a 4x3µm protozoan with cilia extending from the margins, moderately electron-dense grainy cytoplasm with multiple 500nm electron lucent bodies and two moderately electron-dense nuclei. (Photo courtesy of: Wake Forest School of Medicine Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157 www.wakehealth.edu)

present in the nuclear genome. Mitosomes do not generate ATP through the process of oxidative phosphorylation similar to other eukaryotes. Energy generation in these species is largely a process of anaerobic glycolysis with the conversion of glucose to pyruvate occurring in the cytosol, and further oxidation of pyruvate occurring in the hydrogenosome.¹

An extremely important component of this



Heart, rhesus monkey: TEM image of the pericardial fat: An ~11 μ m diameter macrophage, with a stippled electron-lucent cytoplasm and an irregular 5.5x2 μ m electron-dense nucleus contains two 3x4 μ m protozoa as described in figure 4. One protozoal profile has a recurring flagellum arising from the edge of the nucleus. (Photo courtesy of: Wake Forest School of Medicine Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157 www.wakehealth.edu)

particular case is the immunosuppression required for systemic infection by the offending diplomonad. The hematopoietic syndrome is a well-recognized subsyndrome of acute radiation exposure (along with the gastrointestinal and cerebrovascular subsyndromes).⁶ Lymphocytes are extremely sensitive to ionizing radiation and rapidly disappear from the circulation following whole-body irradiation. This is followed by neutrophil loss and then finally platelet loss over the course of several days as existing populations are used up and not replaced by the damaged marrow.⁶ At this point, impaired immunity may result in a wide range of opportunistic infections, or the animal may succumb to fatal hemorrhage as a result of thrombocytopenia.⁶

References:

1. Adam R. Biology of *Giardia lamblia*. Clin Microbiol Rev 2001; 14(3):447-445.
2. Bailey C, Kramer J, Mejia A, MacKey A, Mansfield KG, and Miller AD. Systemic Spironucleosis In two immunodeficient

rhesus macaques (*Macaca mulatta*). Vet Pathol. 2010;47(3):488–494.

3. Baker D. Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. Clin Microbiol Rev. 1998;11:231-66.

4. Boorman GA, Lina PHC, Zurcher C, and Nieuwerkerk HTM. *Hexamita* and *Giardia* as a cause of mortality in congenitally thymusless mice. Clin Exp Immunol. 1973;15:623–627.

5. Fain MA, Karjala Z, Perdue KA, Copeland MK, Cheng LI, and Elkins WR. Detection of *Spironucleus muris* in unpreserved mouse tissue and fecal samples by using a PCR assay. J Am Assoc Lab Anim Sci. 2008;47:39–43.

6. Gulani J, Koch A, Chappell MG, Christensen CL, Facemire P, Singh V, Ossetrova NI, Srinivasan V, Holt RK. Cercopithecine herpesvirus-9 (Simian varicella virus) infection after total-body irradiation in a rhesus macaque. *Comp Med* 2016; 66(2):150-153

7. Jackson GA, Livingston RS, Riley LK, Livingston BA, and Franklin CL. Development of a PCR assay for the detection of *Spironucleus muris*. J Am Assoc Lab Anim Sci. 2013;52(2):165-170.

8. Joint Pathology Center Veterinary Pathology Services Wednesday Slide Conference: Conference: 19 - 2011 Case: 03 Colon – Macaque. c2011. Cited 2017 June 5. Available from: https://www.askjpc.org/wsc/wsc_showconference.php?id=492

9. Jørgensen A, Sterud E. Phylogeny of *Spironucleus* (Eopharyngia: Diplomonadida: Hexamitinae) Protist. 2007;158:247–254.

10. Paull GC MR: Spironucleus vortens, a possible cause of hole-in-the-head disease in cichlids. *Diseases of Aquatic Organisms* 2001;45:197-202.

11. Poynton S. Diplomonad (Hexamitid) Flagellates: Diplomonadiasis, Hexamitosis, Spironucleosis. In: AFS-FHS (American Fisheries Society-Fish Health Section), FHS Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, 2007 ed., AFS-FHS, Bethesda, MD.

12. Sterud E, Atle Mo T, Poppe TT. Systemic spironucleosis in sea-farmed Atlantic salmon *Salmo salar*, caused by *Spironucleus barkhanus* transmitted from feral Arctic char *Salvelinus alpinus*. *Dis Aquat Org.* 1998;33:63-66.

13. Sterud E, Poppe TT, Bornø G. Intracellular infection with *Spironucleus barkhanus* (Diplomonadida, Hexamitidae) in farmed Arctic char *Salvelinus alpinus*. *Dis Aquat Org.* 2003;56:155–61.

14. Wood AM, Smith VH. Spironucleosis (Heramitiasis, Hexamitosis) in the ring-necked pheasant (*Phasianus colchicus*): Detection of cysts and description of *Spironucleus meleagridis* in stained smears. *Avian Dis.* 2005;49:138–143.

CASE II: NCDS-2203/1003-17 (JPC 4067571).

Signalment: 10-12 week old male Sprague-Dawley rat (*Rattus norvegicus*, SD:CrI)

History: This animal was a control animal in a 1 week study for an oncology agent.

Gross Pathology: Dozens of petechiae, ecchymoses and pinpoint to 1.4mm diameter, soft, yellow-tan foci and were scattered throughout the parenchyma of both lungs. Additional petechiae and ecchymoses were scattered throughout the left lateral lobe of the liver and urinary bladder. The epicardium of the right ventricle was irregularly thickened by off-white opaque material.

Gross Pathology: None.

Laboratory results: Ophthalmic examinations were not performed in this study.

Microscopic Description:

Eye: Unilaterally, a locally extensive area spanning the central to peripheral retina, there is thinning of the retina characterized by vacuolation of the photoreceptor layer, shortening and loss of the photoreceptor layer, decreased cellularity to loss of the outer nuclear layer and occasional infiltrating macrophages.

Contributor's Morphologic Diagnoses:

Eye: Unilateral, focal, mild retinal degeneration, photoreceptor layer/outer nuclear layer



Eyes, rat. Sections of two globes are presented for examination. (HE, 4X)

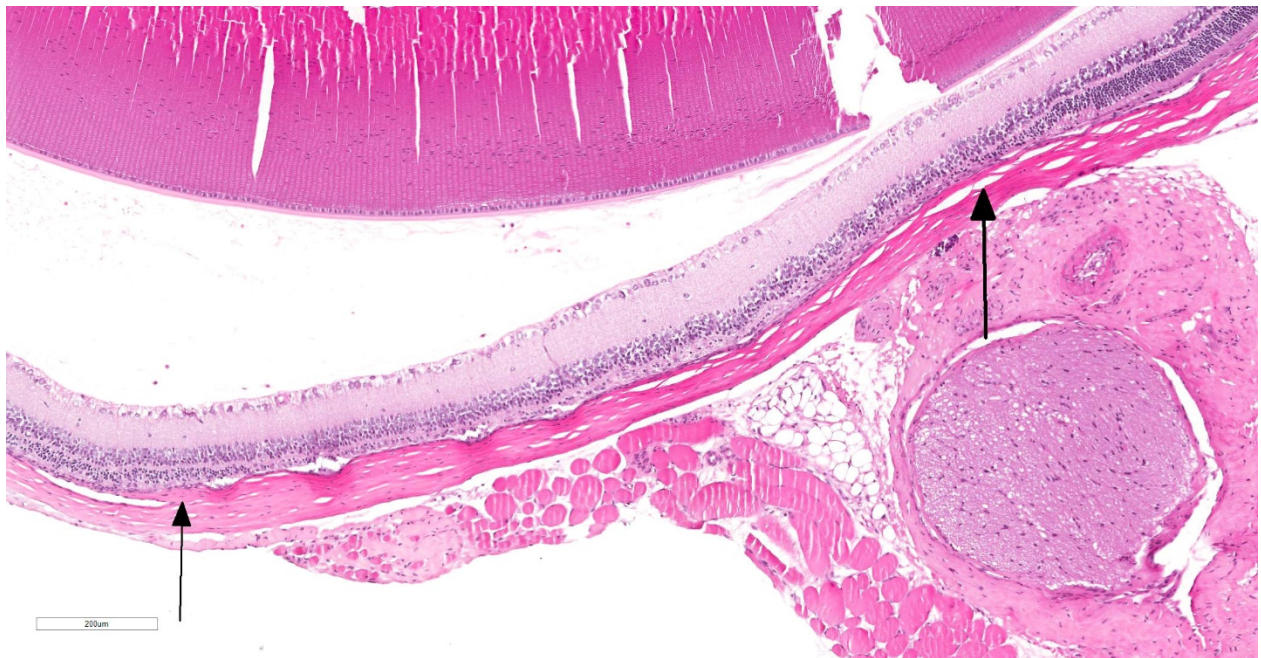
Contributor's Comment: Spontaneous or background retinal findings in the Sprague-Dawley (SD) rat have been attributed to heritable and environmental factors, as well as aging. These findings may be present unilaterally or bilaterally. Spontaneous retinal findings may include dysplasia, dystrophy, and degeneration. Dysplasia is defined as disorganization of the sensory retina with or without degeneration and is also known as "linear retinopathy" in the rat. This is the most common background finding in SD rats greater than 11 weeks of age with 3% incidence in one study.¹ Dystrophy is defined by photoreceptor and/or retinal pigmented epithelium alterations with variable time to onset and progression.

Degeneration is a non-specific term and can encompass a variety of changes in the retina. In the context of spontaneous changes associated with aging, degeneration is defined by decreased numbers of photoreceptor cells and thinning of the outer nuclear layer especially in the peripheral

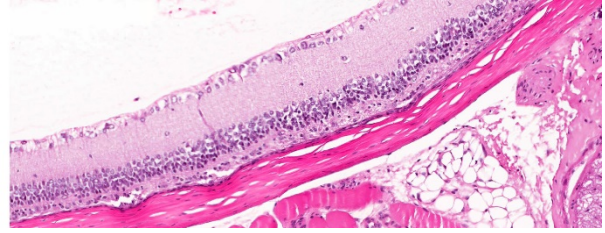
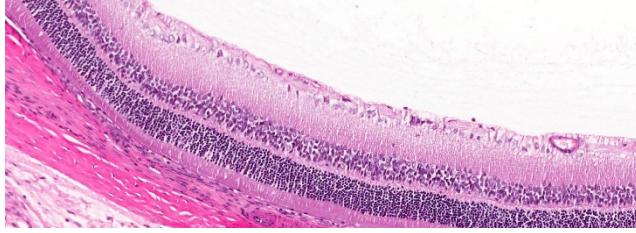
retina. Degeneration associated with excess light exposure is generally more prominent in the central retina and in albino animals. The development of retinal photoxicity can be influenced by a number of factors including gender, light wavelength and diet.² The rate of spontaneous degeneration in SD rats over a broad age range (6 to 24 months, males and females) in one study is 3.7% .⁴ An understanding of the background incidence of retinal degeneration is important since this is the most common manifestation of retinal toxicity. Retinal toxicity can appear microscopically similar to spontaneously occurring retinal degeneration so the incidence and severity must be evaluated to determine the relevance of the finding in given treatment group.²

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Eye, rat. There is a segmental area at the edge of the central retina (delimited by arrows) in which the outer nuclear layer as well as the photoreceptor layer is lost. (HE, 200X)



Eye, rat. Comparison of normal retina (left) and area of retinal degeneration with subtotal loss of the outer nuclear layer and the photoreceptor layer (right). (HE, 400X)

JPC Diagnosis: Retina: Degeneration of the outer nuclear and photoreceptor layer, focal, severe.

JPC Comment: The lesion described above appears to be consistent with “linear retinopathy” – one of the more frequently observed lesions in the rat retina in toxicologic studies, and reportedly prevalent in the Sprague-Dawley rat. This lesion was first reported in 1975 by Schardein et al, under the name “retinal dystrophy” and has also been recorded in the literature as “retinal or retinochoroidal degeneration and atrophy”, “retinal dysplasia/dystrophy”, or “choroidal defect” in Sprague Dawley rats. While most commonly reported in SD rats, it has also been categorized in Crl:CD BR rats.²

This particular lesion, usually unilateral, is the most frequently observed change in rats from 7-10 weeks of age, achieving maximum frequency (around 3%) in animals from 11-14 weeks of age. This frequency appears to be maintained until 110 weeks of age. Fundoscopic examination of affected rats reveals a sharply demarcated, pale, linear area of pallor in varying locations. Histologically, these lesions result in abutment of the inner nuclear layer directly on the underlying choroid or uvea, much as seen in this case.² Early reports describe a 38% incidence of diffuse lesions, which appears rare in subsequent studies and may

simply reflect evolving diagnostic criteria over time. The earliest study in SD rats theorized the possibility of retinal reattachment as a cause for this particular lesion; although other causes, such as genetic or environmental factors have not been ruled out.²

Another form of retinal degeneration not discussed by the contributor is associated with aging. Age-related degeneration includes retinal thinning with a loss of nuclei in the outer and inner nuclear layers, fusion of the nuclear layers, hypertrophy of the retinal pigmentation and possibly migration of RPE cells or macrophages into the sensory retina. Changes are noticed first in the RPE, and often in the peripheral retina, as it is thinner than the central portion.²

Other retinal diseases of a focal nature which may be seen in the retinas of Sprague-Dawley rats. Retinal folds are unilateral lesions that occur at a prevalence of 0.3% at 7-10 weeks of age. They are difficult to see on fundoscopic exam as liner elevations. Histologically, they present as multilayered rosettes.³ Colobomas may be seen at a frequency of approximately 0.5% at 7-10 weeks, and are also generally unilateral. On fundoscopic exam, they often appear as a white discoloration of the fundus in a ventral location, with an abnormal optic disc. Histologically, they present with disordered retinal layers, rosette formation, and ectasia of the optic disk.^{1,3}

References:

1. Hubert M.F., Gillet J.P., Durand-Cavagna G. (1994). Spontaneous retinal changes in Sprague Dawley rats. *Lab Anim Sci.* Dec;44(6):561-7.
2. Render J.A., Schafer K.A. and Altschuler R.A. (2013). Special senses: Eye and ear. In *Toxicologic Pathology: Nonclinical Safety Assessment* (P.S. Sahota, J.A. Popp, J.F. Hardisty and C. Gopinath, eds) pp. 942-948. CRC Press, Boca Raton.
3. Render, J. The eye in safety studies – ocular conditions and issues. C.L. Davis and S.W. Thompson Foundation, *NE Division Symposium on Toxicologic Pathology of the Eye.* <https://www.cldavis.org/cgi-bin/download.cgi?pid=331>.
4. Weisse I. (1994). Aging and Ocular Changes. In *Pathobiology of the*

CASE III: 13004 (JPC 4048861).

Signalment: 8 weeks, male, Wistar/Crlj, *Rattus norvegicus*, rat

History: The rat was administered alloxan (50mg/kg) by an intra-vein injection for single time at the 7 weeks. The rat lost its body weigh gradually after injection. Both the food intake and the urine volume were also less than other diabetic rats. It was sacrificed at the 8 weeks.

Gross Pathology: Both kidneys were enlarged at the time of necropsy.

Laboratory results:

General parameters and blood glucose (BG)

time	Purchased day	injective day	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BW(g)	237.3	239.6	/	232.4	/	/	/	/	202.4

Food Consumption(g)	23.38	13.36	10.55	1.51	3.09	5.76	9.95	14.45	
Urine volume (ml)	28	28	42	22	22	32	26	36	56
BG (mg/dl)	92	/	418	/	/	/	/	/	455

Microscopic Description:

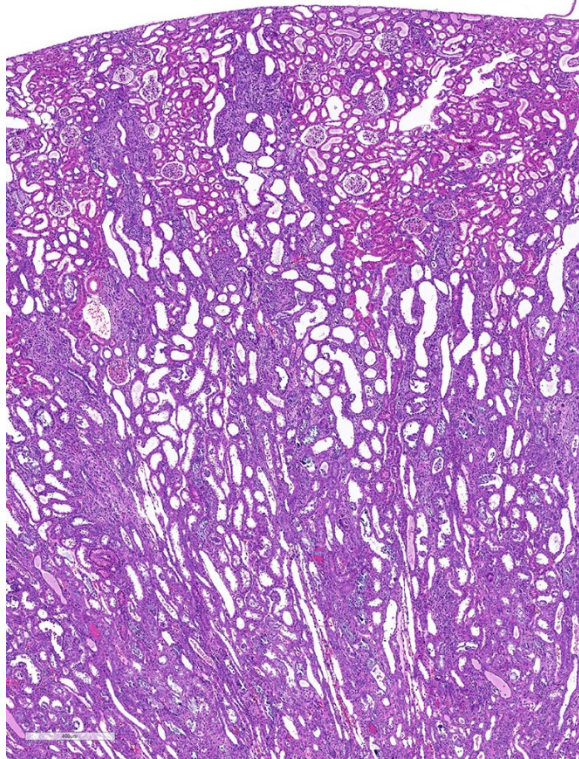
Both two kidneys showed similar lesions. Many dilated and occluded tubules were segmentally observed in inner cortex and outer medulla. Degeneration and necrosis of tubular epithelial cells were seen with/without tubular obstruction by cell debris and crystal/mineral. Dilated tubules were lined with flattened- and attenuated-epithelium with basophilic cytoplasm. In the interstitium around degenerated tubules, multinuclear giant cells, macrophages and lymphocytes infiltrated with fibrosis. Some multinuclear giant cells formed granuloma containing the crystal and mineral. The crystals and minerals located mainly inside the tubules, and some occluded the tubular lumen. In some tubules, they were enveloped by tubular epithelial cells, and tubular basement membrane was disrupted by these crystals and minerals. Mineral was positive for von Kossa's method, but crystal was negative, and both showed non-birefringence with polarized microscopy. Mineral was confirmed as calcium salt. Damaged tubules were mainly the proximal tubules that have brush border and immunopositive for

aquaporin-1, but some were distal tubules that were negative for aquaporin-1 and positive for Na⁺/K⁺ pomp.

Contributor's Morphologic Diagnoses:

Kidney: Granulomatous tubulointerstitial nephritis with tubular necrosis, degeneration and regeneration, and mineralization.

Contributor's Comment: Type 1 diabetes in the rat induced by intravenous injection of alloxan is one of the most common experimental diabetic model which was firstly discovered by Dunn et al. Alloxan induces selectively necrosis of pancreatic



Kidney, rat. There are significant changes within the renal cortex visible within the inner cortex and outer medulla which spares glomeruli and proximal segments of the proximal convoluted tubules. (HE, 20X)

beta cells. Alloxan molecule is structurally similar to glucose, and it can enter beta cells via glucose transporter 2 (GLUT2).² In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalyzed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells.⁴ Nephrotoxicity is a dominating feature of the toxicity of alloxan after systemic administration.⁴ As alloxan is an unstable substance, it disappears within five minutes and almost entirely converted to the relative stable alloxanic acid or alloxanates in plasma and these might exist in the largest amount in urine. Therefore nephrotoxicity is so severe that it causes fatal renal failure in

the animals before diabetes can develop.⁴ The expression of GLUT2 in tubular epithelial cells may explain why the toxins can cause damage to the kidney.⁴

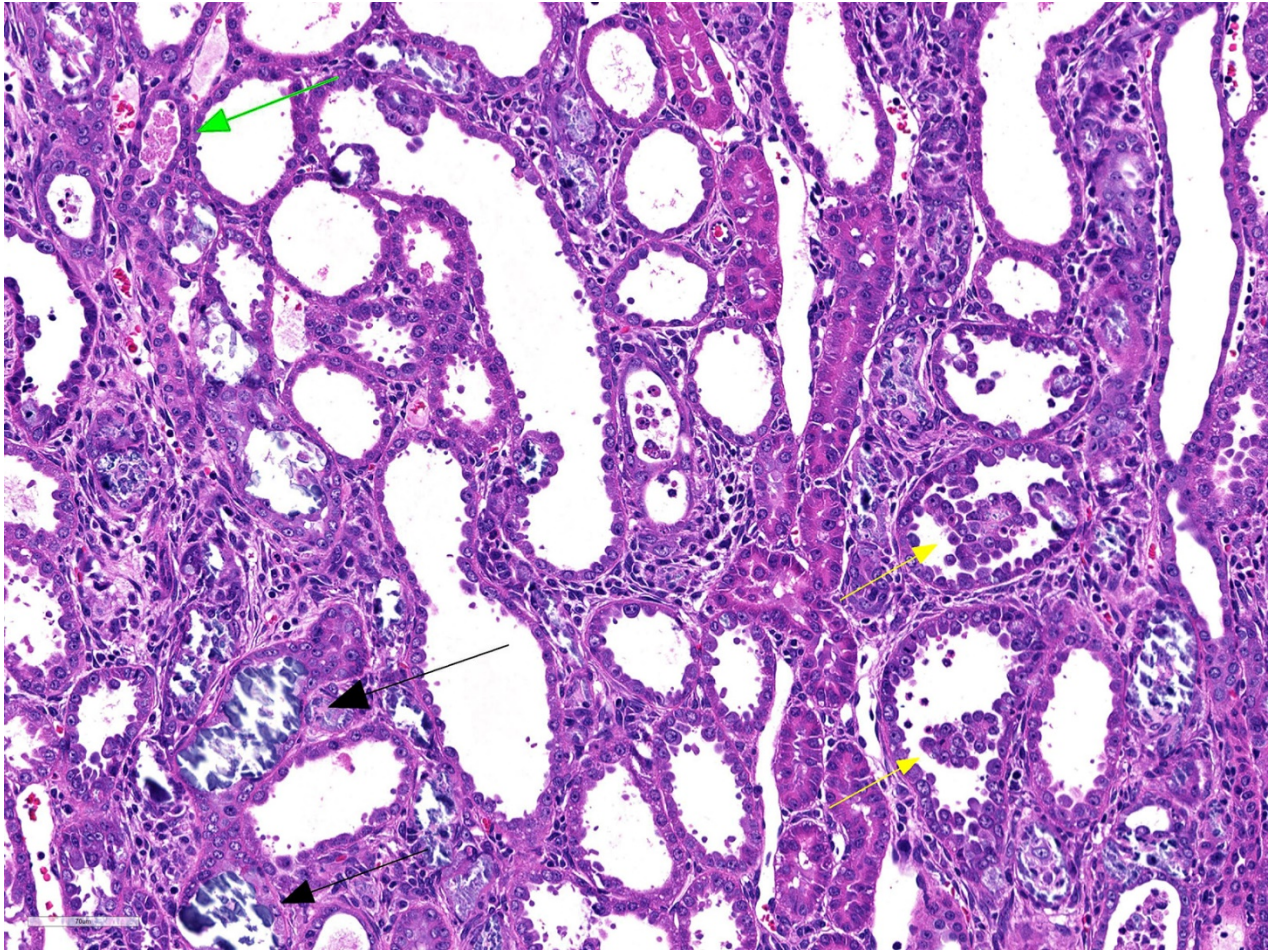
The nephrotoxic changes induced by alloxan include extensive swelling and vacuolar degeneration of renal tubular cells, necrosis of tubular cells with disappearance of brush border and tubular dilation, cellular infiltrates resembling granuloma in the interstitium from the third day to fourteen days.⁵ Histological changes of the kidney observed in alloxan-induced diabetic rats were vacuolar change/ glycogen deposition of the tubular cells and glomerular lesions. Thus, the histologic changes in our case could be differentiated from diabetic change.

Alloxan promotes intratubular mineral deposition and granulomatous interstitial nephritis. These minerals were also localized in the interstitium. The mechanism of translocation of mineral from tubular lumen to interstitium might be similar to that of calcium oxalate crystals¹. Crystal in the tubules is surrounded by tubular epithelial cells and then entered the interstitium through disrupted basement membrane of the tubules and then engulfed by macrophages to form foreign granuloma.

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JPC Diagnosis: Kidney, distal convoluted tubules and collecting ducts: Tubular degeneration, necrosis and regeneration, diffuse, severe, with granulomatous tubular interstitial nephritis, rare intratubular crystals, and marked tubular mineralization.

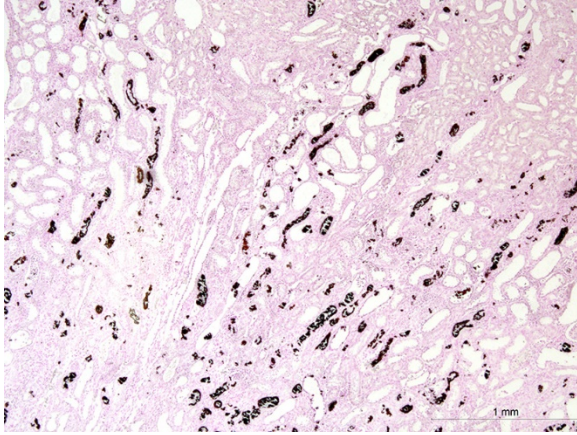


Kidney, rat. A wide variety of changes affect tubules and the interstitium, to include tubular ectasia with epithelial attenuation with granular and cellular casts (green arrow), marked intraluminal proliferation of the tubular epithelium, and the formation of intraluminal oxalate crystals. The interstitial is expanded by variable combinations and concentrations of lymphocytes, plasma cells, histiocytes and neutrophils, collagen, and plump fibroblasts. (HE, 325X)

Conference Comment: Alloxan, a pyrimidine derivative is one of the world's oldest named organic compounds. It was originally derived by the oxidation of uric acid by nitric acid, with early investigators using various sources of uric acid, including human kidney stones as well as boa constrictor excrement (which contains up to 90% ammonium acid urate) The compound was named by Wohler and von Leibig in 1838, who began with the synthesis of urea in 1828, then of uric acid, and some additional 13 derivatives of uric acid of which alloxan is one.⁴ In another one of those cruel twists of fate over naming compounds, Wohler and Leibig were previously aware of similar

research conducted in 1818 by Brugnatelli (working in Italy and publishing in Italian) who created the compound almost two decades previously and had named it "erythric acid" as a result of the red staining it caused on his fingers. The word "alloxan" is derived from a combination of "allantoin", and oxalic acid. A number of reductive products from alloxan exist, including dialuric acid (also diabetogenic in animals), and an anhydrous dimer, alloxantin (produced by the partial reduction of alloxan with hydrogen sulfide.)⁴

As discussed by the contributor, alloxan's similarity to glucose allows it to be taken up

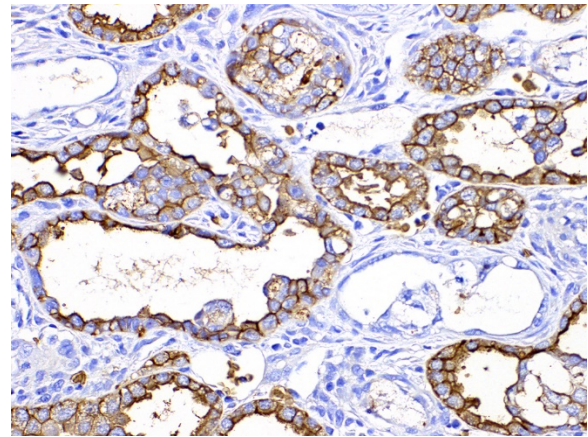


Kidney, rat. A Von Kossa stain demonstrates the prevalence of the mineral within tubules. (HE, 100X 181X) (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan <http://www.setsunan.ac.jp/~p-byori/>)

by GLUT2 glucose transporters in pancreatic beta cells, as well as renal tubular epithelium, resulting in the characteristic necrosis in both cell types, and ultimately “alloxan diabetes” and a change in the kidney initially referred to as “cortical sponge kidney”⁵ (an appellation that like erythric acid, has fortunately not survived the test of time.) Without its structural similarity to glucose, alloxan would not be able to penetrate the cell membrane as it is not lipid soluble.

The basic mechanism of action of alloxan is its generation of reactive oxygen species and selective necrosis of beta cells. The reduction of alloxan within beta cells to dialuric acid results in the generation of the superoxide radical as well as hydrogen peroxide, and in the presence of iron, hydroxyl radicals via the Fenton reaction.³ In addition, alloxan also inhibits glucokinase, inhibiting glucose-induced insulin secretion. This action occurs within a minute of alloxan administration, following alloxan’s almost immediate inhibition of glucose oxidation and generation of ATP necessary to stimulate insulin release from beta cells.³

Another commonly used diabetogenic agent is streptozotocin, a chemotherapeutic alkylating agent and antineoplastic, which was first identified as a diabetogenic agent in 1963.³ This nitrosourea analogue exhibits its effects of beta cells through methylation, or a damaging transfer of a methyl group from streptozotocin to the DNA and other protein molecules of the beta cell following selective uptake through the GLUT2 glucose transporter.³



Kidney, rat. Damaged tubules stain immunopositively for aquaporin. (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan <http://www.setsunan.ac.jp/~p-byori/>)

References:

- 1 de Water R, Noordermeer C, van der Kwast TH, Nizze H, Boeve ER, Kok DJ, Schroder FH: Calcium oxalate nephrolithiasis: effect of renal crystal deposition on the cellular composition of the renal interstitium. *Am J Kidney Dis* **33**: 761-771, 1999
- 2 Elsner M, Tiedge M, Guldbakke B, Munday R, Lenzen S: Importance of the GLUT2 glucose transporter for pancreatic beta cell toxicity of alloxan. *Diabetologia* **45**: 1542-1549, 2002

- 3 Lenzen S: The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* **51**: 216-226, 2008
4. McLetchie NGB. : Alloxan diabetes: a discovery albeit a minor one. *J Rou Coll Physicans Edinb* 2002; 32:134-142.
5. Vargas L, Friederici HH, Maibenco HC: Cortical sponge kidneys induced in rats by alloxan. *Diabetes* **19**: 33-44, 1970

CASE IV: 16-10769 (JPC 4085317).

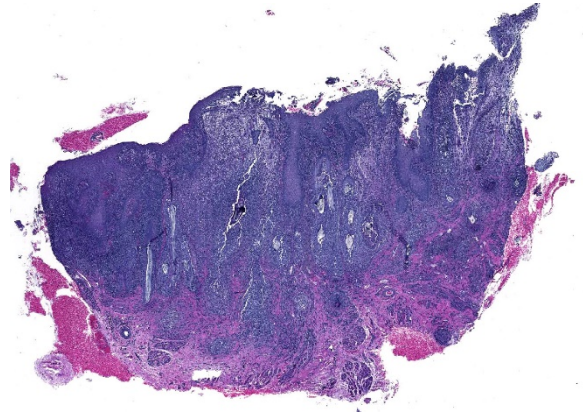
Signalment: 3-year-old, neutered male, mixed breed dog.

History: A 3-year-old mixed breed, neutered male dog had a 6-month history of glossitis with firm raised plaques affecting the proximal third of the tongue. The animal initially responded to prednisone and antibiotic therapy, but improvement stagnated, and two surgical debridements were needed to remove plant material and accelerate the healing process. On follow-up consultation, the dog presented a 95% improvement with only a small focal residual



lesion still present.

Tongue, dog. The dog presented with firm, raised plaques on the dorsal surface of the tongue. (Photo courtesy of: Oregon State University Diagnostic Laboratory, <http://vetmed.oregonstate.edu/diagnostic>)



Tongue, dog. The lingual submucosa is effaced by multifocal to coalescing areas of pyogranulomatous inflammation and granulation tissue which extends deeply into the skeletal muscle and into the overlying hyperplastic and ulcerated mucosa. (HE, 7X)

Gross Pathology: A 3-year-old male dog presented with lesions on the tongue. The rostral third of the tongue had multifocal to coalescing raised, firm and irregular plaques, painful to the touch.

Laboratory results: N/A

Microscopic Description:

Tissue sections of mucous membrane consistent with tongue. Expanding and elevating the submucosa, and infiltrating deeper layers of muscular bundles are severe, multifocal to coalescing, nodular infiltrates of epithelioid macrophages, degenerate neutrophils, and eosinophils surrounding fragments of embedded plant material. Many of the plant fragments have visible spiny projections. The submucosa is multifocally severely edematous with dilated lymphatics and scattered areas of hemorrhage. The surface epithelium is irregularly thickened with scattered intra-corneal pustules, areas of ulceration and is occasionally covered by a 5 to 10 layers of a thick serocellular crust.

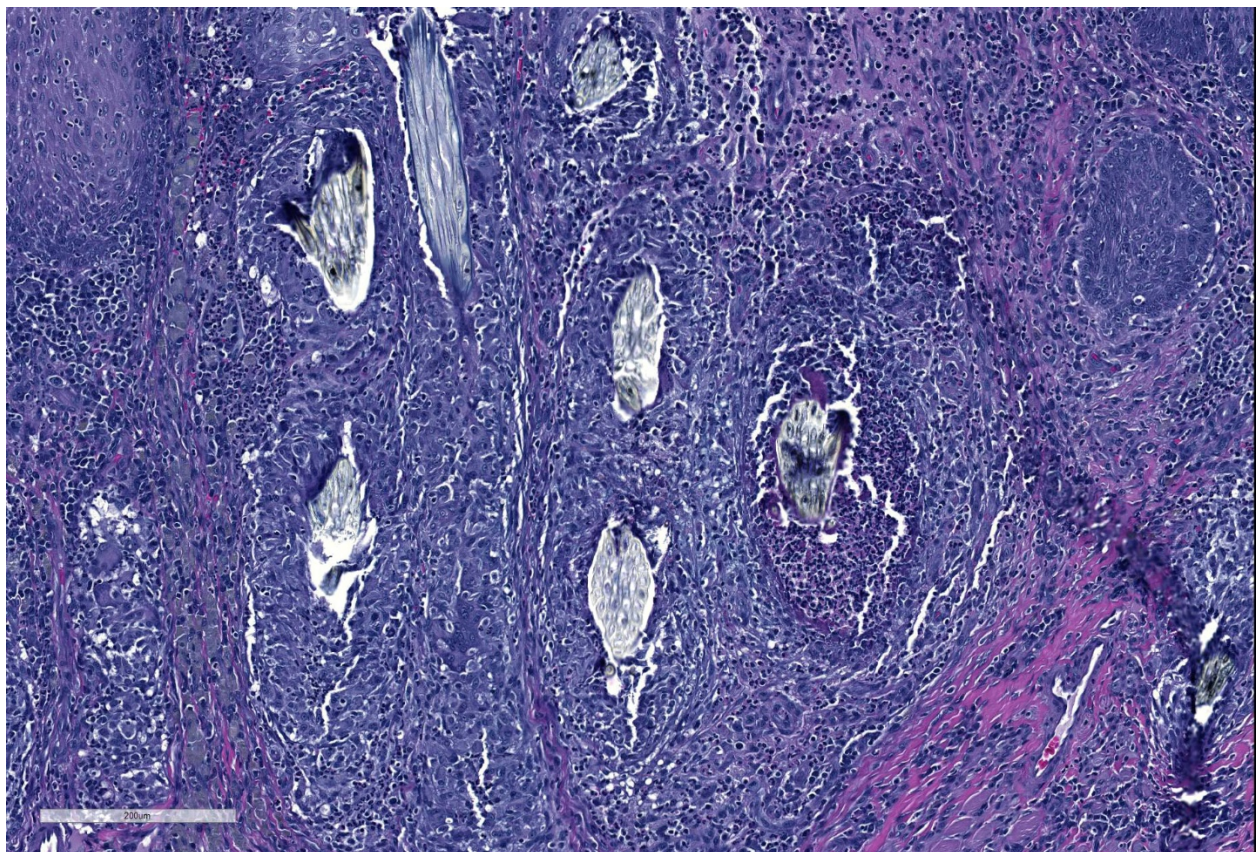
Contributor's Morphologic Diagnoses:

Tongue: Severe, multifocal to coalescing, chronic, pyogranulomatous and eosinophilic

ulcerative glossitis associated with foreign plant material.

Contributor's Comment: Lingual lesions are relatively infrequent in dogs; they mostly consist of neoplasms and glossitis associated with trauma or infections. This case is an example of the latter, a severe granulomatous inflammation associated with foreign plant material. The extension of the lesion combined with spiny projections of the plant is highly suggestive of burdock glossitis ("burr-tongue")¹. *Arctium lappa* L., also known as greater burdock, was introduced to the United States and can be currently found throughout the country, except for a few Southeastern and Southwestern states.⁵

Burdock blooms during the summer (July and August). As a result, there is an increase in the incidence of "burr glossitis" particularly in long haired dogs; plant structures tend to accumulate and stick to the coat around the mouth. The flower has a pappus with numerous exposed bristles with sharp hooks that firmly stick to clothes and animal coats.¹



Tongue, dog. Coalescing pyogranulomas are centered on embedded fragments of plant material with evenly spaced barbules. Some have cores of degenerate neutrophils immediately surrounding the plant material (right). (HE, 145X)

Although *Arctium* sp. are the predominant etiology for this type of lesion, other plants such as cactus glochids and *Setaria* sp. have also been associated with burr-tongue in multiple species.⁴ Different structures of the plant such as burrs, fibers or quills can perforate the tongue and lodge itself in deeper layers of connective or muscular tissue, eliciting an inflammatory response. Oral lesions most often occur on the tip and edges of the tongue, anterior parts of the upper lip and gum, and may also affect the philtrum. Clinical signs vary according to the severity of inflammation and the number of lodged bristles, but they include salivation, anorexia, drooling with mild oral discomfort, halitosis, sensitive mouth, and polydipsia.⁴

Differential diagnosis in these cases include the ingestion of caustic chemicals leading to ulcerative lesions, and ulcerated oral neoplasms such as squamous cell carcinoma, melanoma, and granular cell tumor.

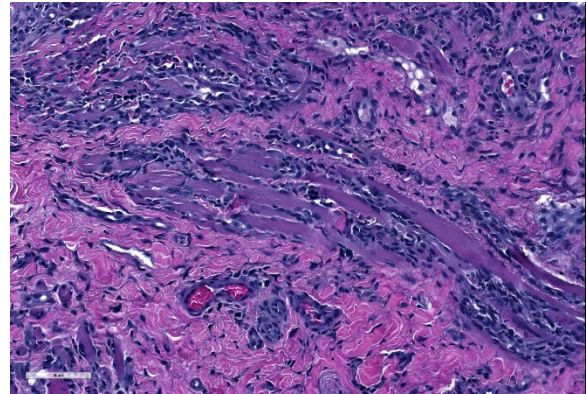
Contributing Institution:

Oregon State University Diagnostic Laboratory
<http://vetmed.oregonstate.edu/diagnostic>

JPC Diagnosis: Tongue: Glossitis, pyogranulomatous and ulcerative, multifocal to coalescing, chronic, with granulation tissue, and abundant plant material.

JPC Comment: The veterinary literature actually contains few reports of plant-induced stomatitis, and the human literature apparently contains none at all (at least based on a very recent Pubmed search.)

“Burr tongue” is a condition which is apparently well-known to practitioners in areas in which *Arctium* sp. grows widely, and is likely a concomitant lesion in animals presented for other complaints.⁴ Clinical signs of careful and slow eating and drinking



Tongue, dog. Atrophic skeletal muscle fibers are surrounded by dense collagen, plump fibroblasts, and abundant perivascular lymphocytic inflammation. Some have cores of degenerate neutrophils immediately surrounding the plant material (right). (HE, 264X)

may not be noticed by the owner. Volcanic ulcers with necrotic centers on the tip and edges of the tongue may require deep curetting or excision to remove the plant scales and the granulation tissue that they are embedded in, and this procedure may need to be repeated several times.⁴

Setaria sp., another common genus of plants referred to as “foxtails” or “bristlegrass”, is well known for causing ulcerative stomatitis in horses when unwittingly incorporated into forage hay. Veterinarians may be consulted for “outbreaks” of stomatitis that may mimic vesicular diseases of the horse, such as vesicular stomatitis, but careful history often reveals the introduction of a new hay source.⁵ *Setaria* stomatitis results in varying degrees of gingivitis, ranging from mild periodontal swelling to marked ulceration, as well as that of labial mucosa in contact with the affected gingiva. Similar, but more severe lesions throughout the oral cavity have also been identified in horse and experimentally in cattle fed a Satu variant of triticale hay in Australia.² Affected animals became totally anorexic and displayed submandibular edema due to the severity of ulcerative lesions throughout the pharynx. A single case report of migrating awns from barley clusters

was reported to cause lingual arteritis, meningoencephalitis, and uveitis in a two-year-old heifer.

Histologically, the lesions of this and other plant awns, such as glochids from prickly pear cacti and foxtail awns from *Setaria* sp. have been reported to be identical both with regard to the characteristic chronic and pyogranulomatous inflammation as well as the appearance of the plant material itself. While the barbs on the bristles of these plants may be oriented either anteriorly or posteriorly, this bit of identifying information is generally useful only in examining the plants themselves under a dissecting microscope and not in examining tissue histologically.

References:

1. Georgi, M. E., Harper, P., Hyypio, P. A., Pritchard, D. K., & Scherline, E. D. Pappus bristles: the cause of burdock stomatitis in dogs. *The Cornell veterinarian*, 1982;72(1), 43-48.
2. McCosker, JE, Keenan DM. Ulcerative stomatitis in horses and cattle caused by triticale hay. *Austr Vet J* 1983; 60:259.
3. Sorden SD, Radostits OM. Lingual arteritis, multifocal meningoencephalitis and uveitis induced by barley spikelet clustes in a two-year-old heifer. *Can Vet J* 1996; 37:227-229.
4. Thivierge, G., 1973. Granular stomatitis in dogs due to Burdock. *The Canadian Veterinary Journal*, 14(4), p.96.
5. Turnquist, SE, Ostlund EN, Kreegar JM, Turk JR. Foxtail-induced ulcerative stomatitis outbreak in a Missouri stable. *J Vet Diagn Invest* 2001: 13-238-240.

6. United States Department of Agriculture: (USDA)

<http://plants.usda.gov/core/profile?symbol=ARLA3>