Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 4

CASE I: 16-02795 (JPC 4087577)

Signalment: Adult (age and gender not specified) little red flying fox (*Pteropus scapulatus*).

History: This bat was in the care of a local wildlife rescue group, when its vocalisations became abnormal, then developed paresis which progressed to grand mal seizures. The bat was euthanased, and tissues submitted to the laboratory for exclusion of Australian Bat Lyssavirus (ABLV).

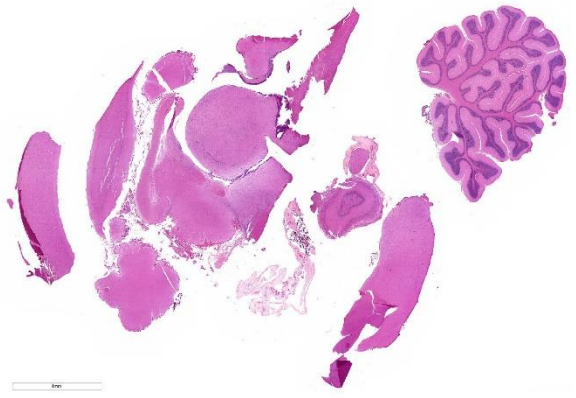
Gross Pathology: No gross lesions observed

Laboratory results: Immuno-histochemistry: Immunohistochemistry using monoclonal (HAM Mab 100) and polyclonal (Poly 663) antibodies raised against rabies nucleoprotein showed diffuse positive staining in neurons throughout the cerebral cortex and brainstem, and in cerebellar Purkinje fibres. ABLV antigen was most concentrated within neurons in the hippocampus, thalamus, and pons.

Microscopic Description:
Brain: Blood vessels throughout the brainstem and cerebrum are surrounded by lymphocytes and fewer plasma cells. Multifocally within the brainstem, thalamus and rarely in cerebellar Purkinje cells, neurons have one to numerous oval, well demarcated, eosinophilic intracytoplasmic inclusions (Negri bodies). The cranial nerve is diffusely infiltrated with many lymphocytes and fewer plasma cells. Meninges in the brainstem are focally expanded with neutrophils and lymphocytes, with fewer plasma cells.

Contributor's Morphologic Diagnosis:

1. Brain; brainstem, cerebrum, and cerebellum: Encephalitis, lymphoplasmacytic, subacute, diffuse, mild, with multifocal intraneuronal intracytoplasmic

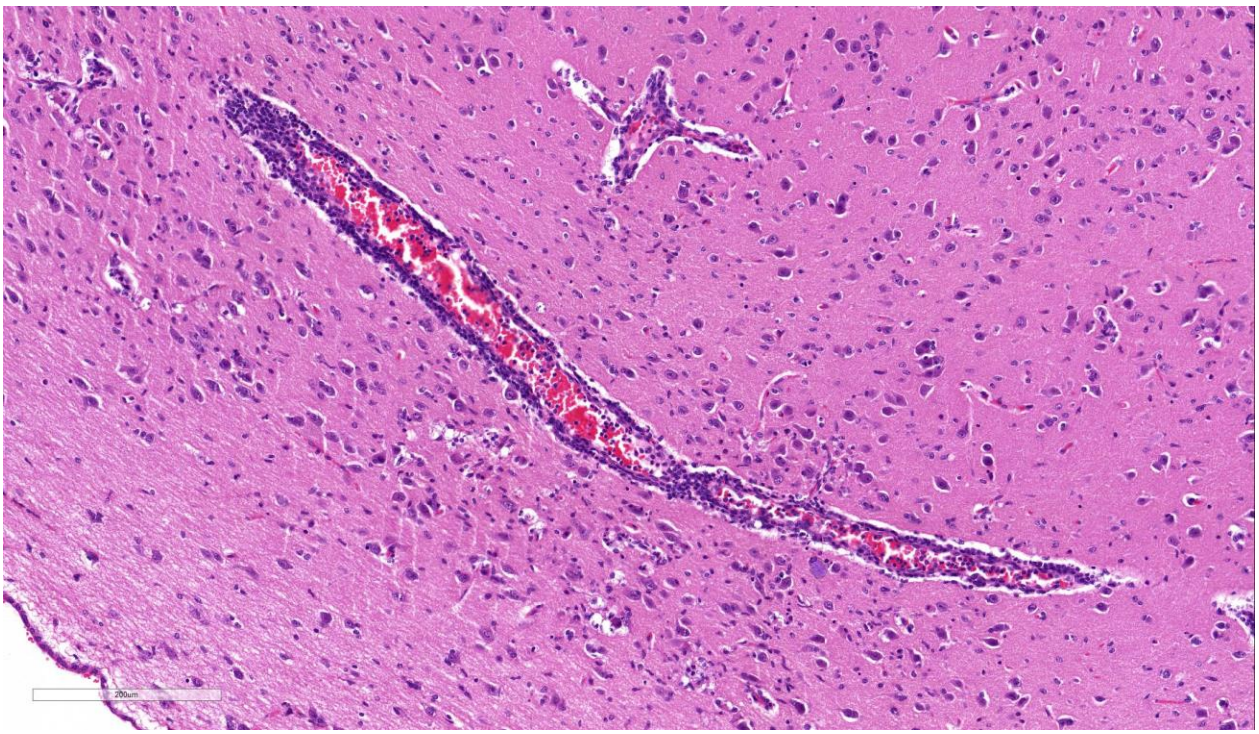


Brain, little red flying fox. The entire brain is submitted for examination. (HE, 8X)

eosinophilic inclusions (Negri bodies)
2. Ganglion: Ganglioneuritis, lymphoplasmacytic, subacute, diffuse, moderate
3. Meninges: Meningitis, neutrophilic and lymphocytic, subacute, focal, mild, with multifocal calcification

Contributor's Comment: Australian bat lyssavirus (ABLV) belongs to the genus *Lyssavirus*, family *Rhabdoviridae*, order *Mononegavirales*.[10] The genus *Lyssavirus* includes seven genotypes: rabies virus (RABV, genotype 1), Lagos bat virus (genotype 2), Mokola virus (genotype 3), Duvenhage virus (genotype 4), European bat lyssavirus 1 (EBLV-1, genotype 5), European bat lyssavirus 2 (EBLV-2, genotype 6), and Australian bat lyssavirus (ABLV, genotype 7). ABLV is most closely related to classical rabies (genotype 1), and vaccination against rabies provides protection against exposure to ABLV. Two variants of ABLV have been described, in pteropid and insectivorous bats.[5,6] Natural infections with ABLV have been described in horses,[11] with affected animals displaying clinical signs similar to those of classical rabies. There have been three reported cases of ABLV infection in Australia, all of which were fatal.[1,4,7,8]

Microscopic lesions typically associated with *Lyssavirus* infections include a nonsuppurative encephalomyelitis, with multifocal gliosis, neuronal degeneration, and rare oval, eosinophilic intracytoplasmic viral inclusions (Negri bodies).[2] The distribution of Negri bodies in domestic animals has been described as occurring most commonly in the hippocampus in carnivores, and in the Purkinje cells of herbivores.[2] Hooper et al. (1999) published a survey of

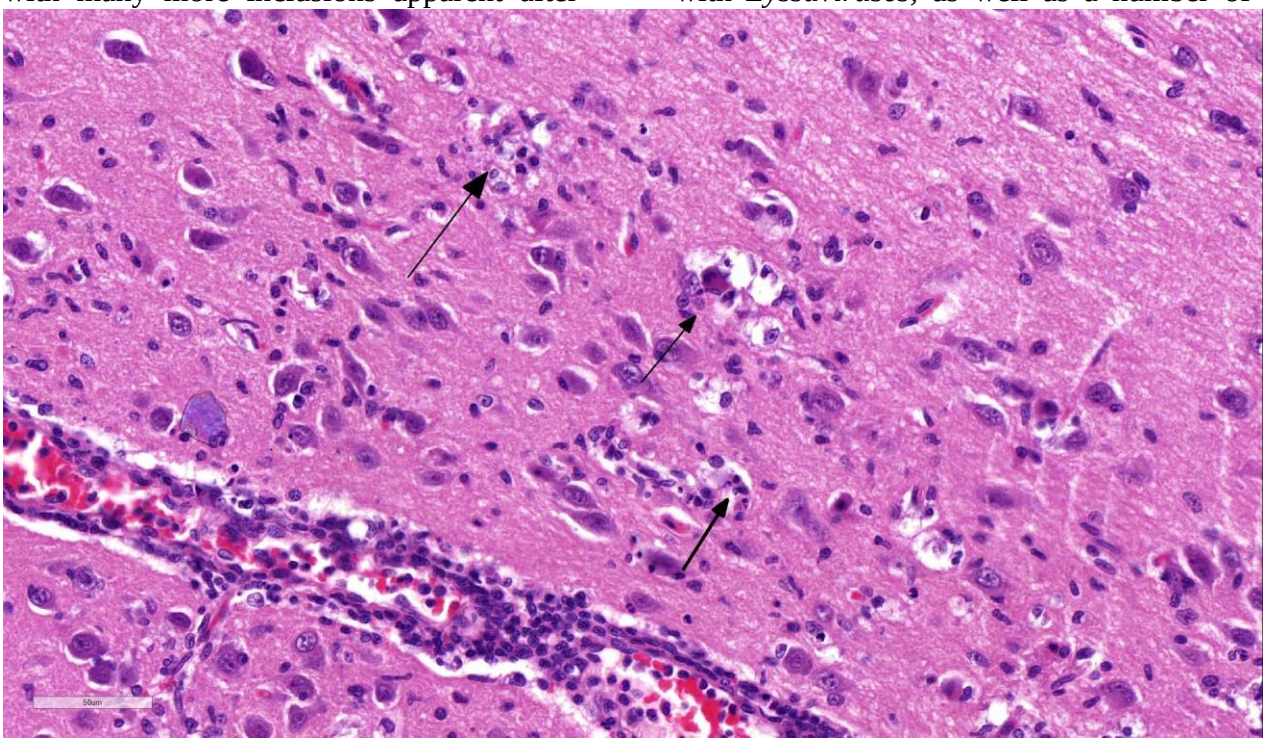


Thalamus, little red flying fox. Multifocally, scattered throughout the brain parenchyma, vessels are cuffed by low to moderate numbers of lymphocytes and rare plasma cells. There is a moderate gliosis and multifocal neuronal necrosis. (HE, 100X)

lesions in the brains of bats that had tested positive for ABLV, and reported lesions in the hippocampus, thalamus and midbrain, and medulla oblongata and pons.[9] The authors observed Negri bodies in only 9 of 21 bats. Immunoperoxidase staining using antibodies raised against rabies viral nucleoprotein revealed a reaction pattern with a distribution similar to that of microscopic lesions. Positive immunohistochemical staining was noted to occur in the absence of lesions. Positive immunohistochemical staining was also reported in the dorsal root ganglia and in ganglia of the alimentary system. There was only loose relationship between staining of Negri bodies on H&E section and the presence of virus in neurons.

In the present case, the relationship between immunohistochemical staining and the presence of Negri bodies was similarly loose, with many more inclusions apparent after immunohistochemical staining. In addition, immunohistochemical staining of intraneuronal inclusions demonstrated a widespread distribution of viral antigen. Neuronal necrosis and gliosis were not features in the current case. This case was unusual in our laboratory with submission of only the fixed brain rather than the whole fresh bat and ABLV exclusion through tissue sampling of swabs from the oral cavity, salivary gland, and brain for PCR.[11] Fresh salivary gland and brain tissues are subsequently sent to the national reference laboratory for direct fluorescent antibody testing, which is the international gold standard diagnostic test. While the results of immunohistochemical testing highlight the relative insensitivity of routine histology as a diagnostic method for *Lyssaviruses* it does demonstrate its ability to secure a positive diagnosis.

Bats are unique in that they are able to coexist with *Lyssaviruses*, as well as a number of



Thalamus, little red flying fox. Higher magnification of the field in fig 1-2. Neurons are shrunken and fragmented and surrounded by microglia (arrows). (HE, 400X)

other viruses that are lethal to most mammals (e.g. Hendra, Nipah, Severe acute respiratory system coronavirus [SARS-CoV], Ebola, Mekala, and Marburg viruses). Research indicates that the Australian black flying fox, *Pteropus alecto*, constitutively expresses interferon alpha genes,[14] and type III interferons that are differentially expressed compared to type I interferons.[13] However, the immunologic mechanisms by which bats are able to tolerate infection with these otherwise lethal viruses are only beginning to be unraveled.

Contributing Institution:
State Veterinary Diagnostic Laboratory
Elizabeth Macarthur Agricultural Institute
Woodbridge Rd
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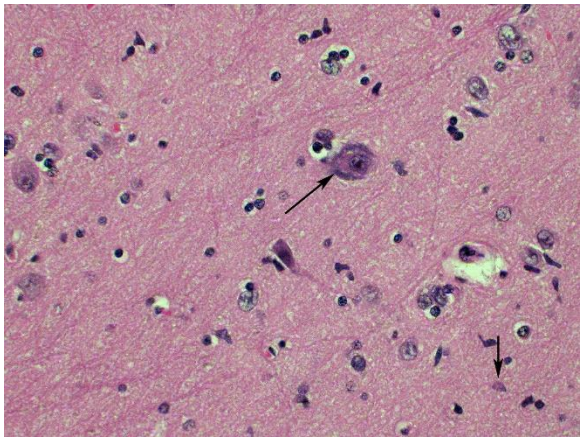
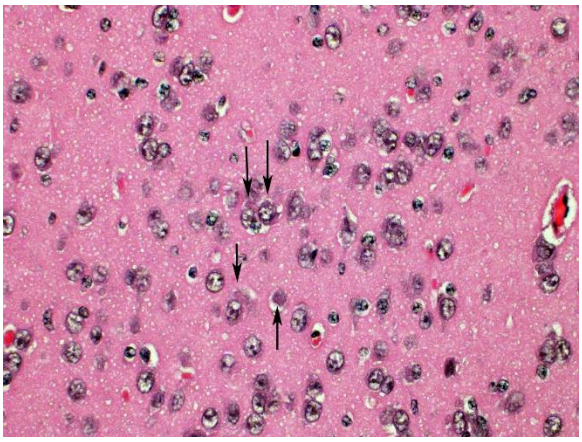
Australia

JPC Diagnosis: Brain: Meningo-encephalitis, diffuse, lymphoplasmacytic, mild with numerous neuronal intra-cytoplasmic viral inclusions.

JPC Comment: The contributor has done an outstanding job describing Australian bat lyssavirus (ABLV) and the peculiarities noted upon comparison of histologic and immunohistochemical evaluation on tissue from infected animals. This case compares favorably with a series of ABLV-infected bats by Hooper et al.[9] discussing the great variability of immunopositivity in neurons in tissues infected with lyssaviruses (similar to that in seen in rabies cases.) To maximize the diagnostic potential of immuno-histochemistry in suspect cases of any lyssavirus, submission (and evaluation) of tissues in addition to cerebrum is highly recommended, to include spinal cord and dorsal root ganglia, adrenal gland, and gastrointestinal tract (inclusions may be found in GI nerve plexes.)[9]

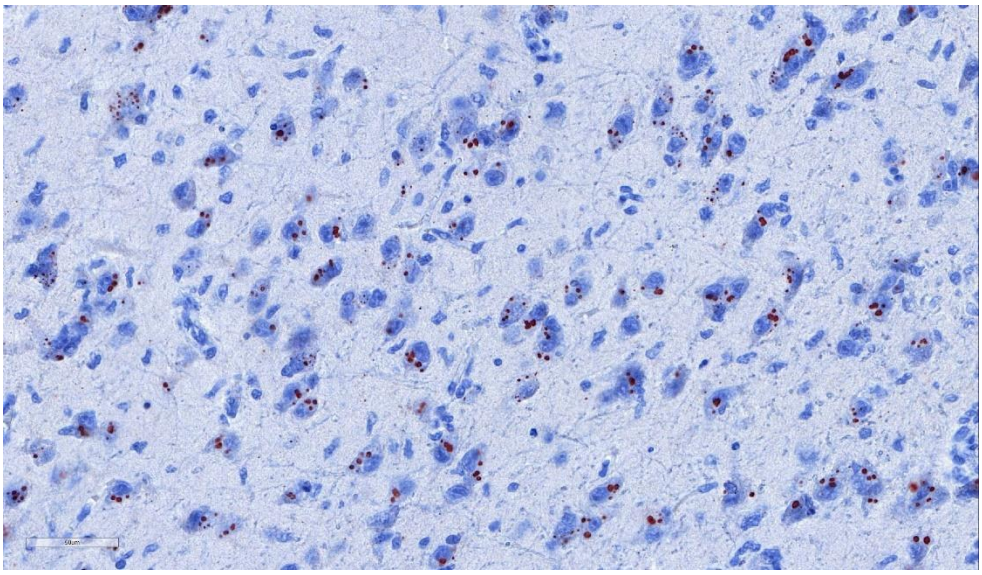


Thalamus, little red flying fox. Neurons often contain multiple intracytoplasmic 1-2um round viral inclusions (Negri bodies). (HE, 400X)



Olfactory cortex, little red flying fox. Neurons contain multiple intracytoplasmic 1-2um round viral inclusions (Negri bodies). (HE, 400X)

As previously noted, ABLV infection in humans is a rare finding, with only three reported cases, but with a mortality of 100%. Infection rates in wild bat population have been estimated at less than 1%, but purported to be markedly increased (5-10%) in sick or injured bats, the ones most likely to come into contact with humans. Like rabies, direct contact is required for zoonotic transmission, and in the documented cases of lyssavirus in humans, the latent period ranged from one month to 27 months.[4]

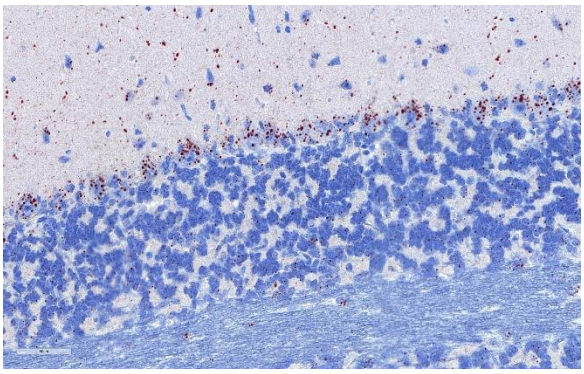


Cerebrum, little red flying fox. While difficult to see on HE, immunostaining for flying fox lyssavirus antigen shows its widespread location in neurons of the cerebrum. (Photo courtesy of: State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Woodbridge Rd. Menangle, NSW 2568 Australia)

Antemortem diagnosis of Australian bat lyssavirus in humans, as in rabies, is difficult. Neuroimaging is usually of little benefit. CSF pleocytosis is mild at best and present in only approximately 60% of cases.[4] Lyssaviral antibodies may appear late in the course of disease or not. The utility of nuchal skin biopsy (considered to be 98% specific) is yet to be verified in human cases of lyssavirus infection, but bat lyssavirus has been shown to cross-react with rabies RT-PCR, so this test, as well as salivary PCR should be considered.[4] Clinically, the three cases of zoonotic bat lyssavirus resembled the encephalitic or "furious" form of rabies, characterized by hyperactivity, agitation, and spasms, especially of the face. As treatment for lyssavirus has not been established, but post-exposure prophylaxis is considered to be protective if offered.[4]

In addition to ABLV and rabies, two types of lyssavirus have been identified in European bats – European bat lyssavirus types 1 and 2 (EBLV-1 and EBLV-2); EBLV-1 is further subdivided into subtypes 1a and 1b. Both types have been reported as causing neurologic rabies-like syndromes in man, as well as cats and sheep.[3]

The distributed slides psed a challenge for participants as few identified lesions in the submitted tissue other than the numerous Negri bodies. The slide posted online at https://www.askjpc.org had an identifiable ara of meningeal inflammation, and the parenchyma in its proximity exhibited gliosis with rare neuronal necrosis and satellitosis (Fig 1-3). The architecture of one of the submitted sections was unfamiliar to the participants; many identified it as hippocampus until Dr. Andrew Cartoceti of



Cerebellum, little red flying fox. Intracytoplasmic inclusions of flying fox lyssavirus are present within neuronal cytoplasm of Purkinje cells as well as neurons of the granular cell layer.(Photo courtesy of: State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Woodbridge Rd. Menangle, NSW 2568 Australia)

the National Zoo identified it as olfactory cortex.

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CASE II: 17-0894 (JPC 4111545).

Signalment: Merino, Female, 12 months old

History: 14 of 130 twelve month old lambs died during a two week period in late Autumn. Sheep were grazing pastures containing witchgrass (*Panicum capillare*)

Forage. Animals had been grazing pastures containing witchgrass (Panicum capillary capillare). (Photo courtesy of: Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, the University of Adelaide, Roseworthy, South Australia, 5371, Australia.

Gross Pathology: Cutaneous photo-sensitization of eyelids, muzzle and coronet bands. Severe generalized jaundice, very swollen yellow and firm liver with enhanced lobular pattern. Dark yellow-brown staining of kidneys.

Laboratory results: None performed.

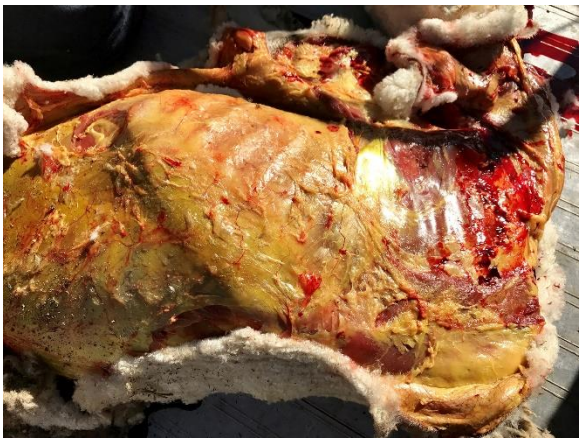
Microscopic Description:
Liver: Lobar borders of liver are rounded. There is mild to moderate infiltration of periportal interstitium by lymphocytes, macrophages, and variable neutrophils, mild to moderate expansion of periportal fibrocollagenous connective tissue, and mild to moderate bile duct hyperplasia. Within periportal interstitium there are clear acicular clefts (crystal ghosts) surrounded by fibroblasts and collagen. Rare crystal ghosts are present in the bile ducts associated with tubular epithelial degeneration and attenuation, and less often present in the sinusoids. Diffusely, there is moderate hepatocellular swelling characterized by feathery to vacuolar cytoplasm and scattered individual cell necrosis, most notably in periportal regions. Kupffer cells are often enlarged with vacuolar to lightly pigmented cytoplasm, or clear cytoplasm. Bile canaliculi are sometimes distended by light brown-orange pigment (bile plugs).

Microscopic findings in other tissues included myocardial sarcocystosis, moderate to severe neutrophilic bronchointerstitial pneumonia, and renal tubular epithelial degeneration and cytoplasmic pigmentation (bilirubin, other) with pigmented tubular casts.

Sheep, presentation. Affected sheep had facial alopecia and swelling. (Photo courtesy of: Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, the University of Adelaide, Roseworthy, South Australia, 5371, Australia)

Contributor's Morphologic Diagnosis:
Liver: chronic-active mixed cholangio-hepatitis with cholestasis, periportal fibrosis and crystal ghosts (crystal cholangio-hepatopathy).

Contributor's Comment: Crystal-associated hepatopathy and secondary photosensitization in these lambs developed following exposure to witchgrass (*Panicum capillare*) in pasture. No crystals were observed under direct nor polarized light, however acicular clefts (crystal ghosts) were identified in the periportal interstitium, bile ducts and rarely in the sinusoids. The duration that lambs had been grazing this pasture prior to the onset of clinical signs was not recorded in the submission. Onset of hepatotoxicity in sheep experimentally exposed to another *Panicum* species, kleingrass (*Panicumn coloraturum*), was variable, occurring as early as three days to several weeks post initial exposure [1]. Following natural exposure of sheep to *P. schinzii*, the clinical appearance of hepatotoxicity was reported to have occurred within five days of the flock being moved onto affected pastures [2].



Sheep, presentation. Affected sheep had generalized icterus. (Photo courtesy of: Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, the University of Adelaide, Roseworthy, South Australia, 5371, Australia)

Crystal hepatopathy has been reported in ruminants and horses following exposure to *Panicum* grasses 1, as well as *Brachiaria* [3,4], and *Narthecium*[14] grass species. These plants contain steroidal saponins and affected ruminants often develop bile crystals composed of the calcium salts of steroidal saponin glucuronides.[6,8-10]

Hepatic lesions described in natural and experimental *Panicum* grass toxicity in sheep and horses include patchy or scattered

Sheep, liver: Livers were firm, swollen and yellow with an enhanced lobular pattern on cut surface. . Affected sheep had generalized icterus. (Photo courtesy of: Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, the University of Adelaide, Roseworthy, South Australia, 5371, Australia)

hepatocellular necrosis, vacuolation and swelling, nuclear chromatin clumping, presence of birefringent crystals in small bile ducts, bile canaliculi or within sinusoidal phagocytes, fibrosis, bile duct proliferation, and lymphocytic hepatitis.[1,7] Crystals may not always be observed; no crystals were identified in affected livers of horses and sheep following exposure to *Panicum dichotomiflorum*, [7] and this was thought to be to the short exposure time to the grasses (feeding trials only lasted 12 days). In experimental work done elsewhere, sheep grazing kleingrass for less than 10 days did not have observable crystals in the liver [1]. In kleingrass toxicity, crystals are reportedly

Sheep, kidney: Kidneys are icteric with a dark brown cortex (Photo courtesy of: Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, the University of Adelaide, Roseworthy, South Australia, 5371, Australia)

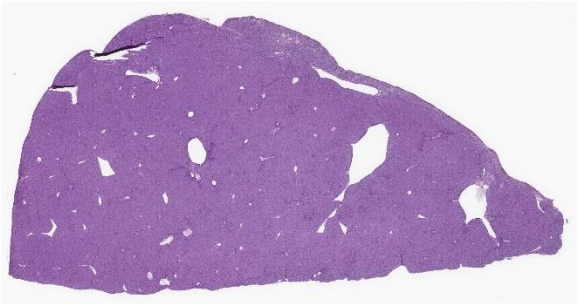
soluble in acidified ethyl alcohol, acetic acid, pyridine, chloral hydrate, and methanol, but not in xylene, petroleum ether, diethyl ether, acetone, water, or cold ethyl alcohol. [1]

Contributing Institution:
Veterinary Diagnostic Laboratory
School of Animal and Veterinary Sciences
The University of Adelaide,
Roseworthy, South Australia, 5371, Australia

JPC Diagnosis: Liver: Cholangiohepatitis, necrotizing and histiocytic, multifocal, mild, with mild bridging portal fibrosis, biliary hyperplasia, subcapsular hepatocellular loss intrahistiocytic and intraductal crystal formation.

JPC Comment: The genus *Panicum* is a large group of grasses containing up to 600 different species of grasses in tropical and temperate regions around the world.[13] The majority may be used as forage for livestock; however a limited number of species such as those mentioned above, may result in crystal associated hepatopathy, with the formation of crystals within bile ducts and resultant hepatic damage as the most important lesion. The biotransformed saponins within these plants are conjugated with gluruconic acid and excreted in the bile, where they may complex with calcium to form an insoluble salt. Photosensitization in affected animals is usually the result of biliary damage and inability to conjugate phytoporphyrins (phylloerythrin), a chlorophyll derivative produced by bacteria in the gastrointestinal tract. Phylloerythrin is a photoactive compound which is excited by ultraviolet light in lightly pigmented and poorly haired areas of the body.

A recent article by Sillman et al[12] details toxicity in 3-month Boer goats clearing a weedy livestock lot. After a month of the lot, affected animals developed icterus and inappetence with marked hyperbilirubinemia, elevated aspartate aminotransferase and variable levels of alanine aminotransferase. Examination of the lot showed a tremendous growth of *Panicum dichotomiflorum*. At autopsy, portal areas were mildly fibrotic and contained clusters of macrophages centered on large, acicular crystals. Mild hepatocellular vacuolation and biliary hyperplasia were also noted. Previous reports of *P. dichotomiflorum* described intoxication in a variety of species, including nursing and juvenile lambs and horses, although many reports note that other animals of various species, breeds, or ages grazing the same forage were unaffected. The report by Sillman et al. also notes the

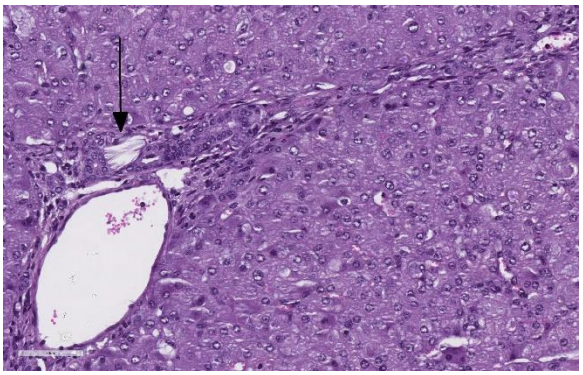


Sheep liver. A section of liver is submitted with enhanced visibility of portal triads and a focal subcapsular scar. (HE,7X)

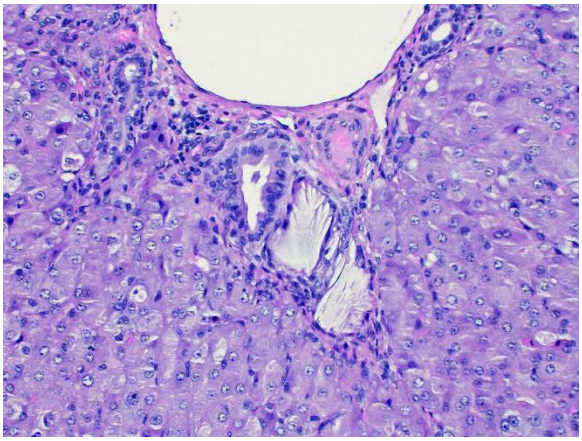
presence of tubular nephrosis in the goat, which had not been previously described. Evidence of photosensitization was not noted in these cases. [12]

Another recent report describes the first instance of hepatogenous photosensitization due to steroidal saponin toxicity in macropods. Steventon et al.[13] describes an outbreak of blindness or photophobia in 95 grey kangaroos in New South Wales, Australia. Affected animals developed icterus, photophobia (abnormal shade seeking behavior), and blindness resulting with severe corneal edema and inflammation and necrotizing dermatitis of the eyelids, pinnae, and forearms. Examination of the liver of affected animals revealed crystal formation in small bile ducts with mild granulomatous inflammation and portal fibrosis. The presence of crystal-associated damage and inflammation with bile ducts prompted examination of the site of the outbreak, which was dominated by a heavy growth of *Panicum gilvum* ("sweetgrass" or "sweet panic".)[13]

Sporidesmin, a plant saponin from spores of *Pithomyces chartarum* which causes a well-known syndrome of photosensitization ("facial eczema") and severe hepatic damage in New Zealand. The excretion of unconjugated sporidesmin in the bile results in oxidative injury to the biliary epithelium, leakage of biliary components into the surrounding tissue, and occasionally necrosis of vessels in adjacent parenchyma.[4] When complemented with concurrent ingestion of saponins of *Tribulus terrestris* a condition known as "geeldikkop" is created, in which crystal formation within bile ducts and within the whitish contents of the gallbladder is noted.[4]



Sheep, liver: Portal areas contain increased profiles of bile ductules and small amounts of collagen. Sheaf-like crystals are present in close proximity to, or within bile ductules. (HE, 315X)



Sheep liver: Higher magnification of crystals within and adjacent to bile ducts. (HE, 400X)

Conference participants were impressed by the significant icterus demonstrated in the gross lesions in this case as compared with the relative lack of significant fibrosis and hepatocellular damage. While information about possible exposure to copper was not mentioned, a JPC-run rhodamine stain was considered unremarkable in this case.

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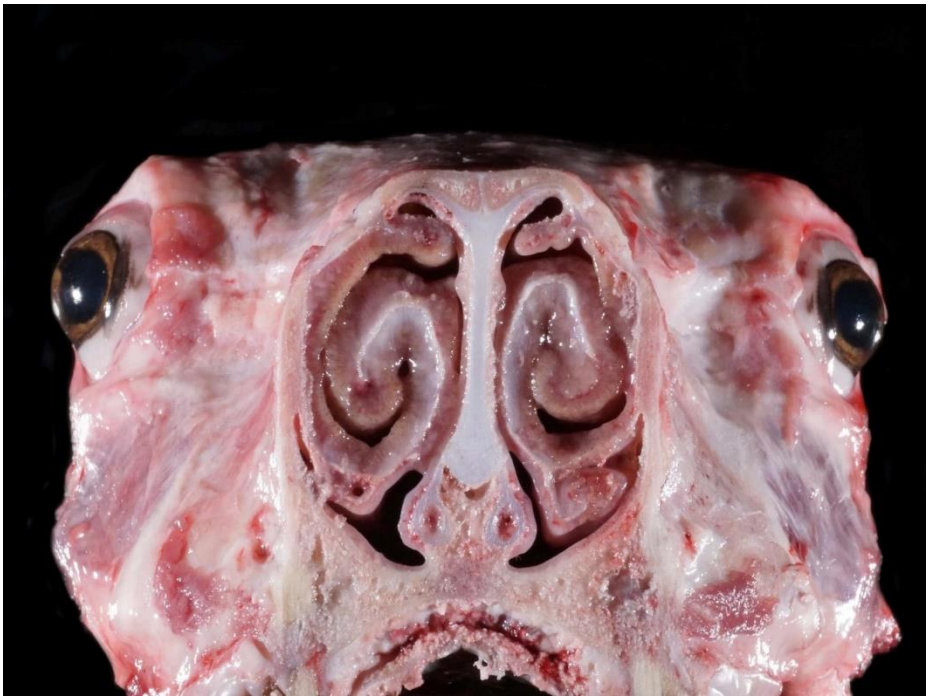
CASE III: UMN VDL (JPC 4116936)

Signalment: 3-year-old ewe (*Ovis aries*) breed unknown.

History: Pesistent nasal discharge and labored breathing. This animal was euthanized.

Gross Pathology: There was a moderate amount of mucoid discharge in the nares. On coronal section, the nasal turbinates



Nasal mucosa, sheep. The mucosa lining the turbinates is diffusely hyperemic and markedly thickened, occasionally occluding the meatus. (Photo courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory.

(conchae) were diffusely and markedly thickened, with a roughened appearance and the meatuses were narrowed.

Laboratory results: Aerobic culture of a nasal swab yielded a 4+ predominant *Salmonella* sp. serotyping of this isolate performed by NVSL identified it as a serotype III 61:k:1,5,7 (*S. enterica* subspecies *arizonae).* Immuno-histochemistry for *Salmonella* sp. showed strong positive immunoreactivity distributed throughout the nasal mucosa and submucosa, with epithelial cells, goblet cells, macrophages, and extracellularly.

Microscopic Description: The mucosa of the nasal turbinate is markedly thickened by polypoid to finger-like projections of hyperplastic epithelial cells and dense infiltrates of plasma cells and lymphocytes admixed with few neutrophils, macrophages, and siderophages within the lamina propria and submucosa. The infiltrates within the lamina propria and submucosa separate and surround hyperplastic seromucous glands, which are often ectatic and contain intraluminal mucin and cellular debris. Glandular and surface epithelial cells often contain intracytoplasmic aggregates of indistinct, 1-2um diameter eosinophilic coccobacilli. There are moderate numbers of intraepithelial neutrophils, lymphocytes, and macrophages. There is multifocal follicular lymphocid hyperplasia within the submucosal lymphoid tissue and there are few coalescing pyogranulomas within the lamina propria and submucosa, which replace and compress adjacent glandular

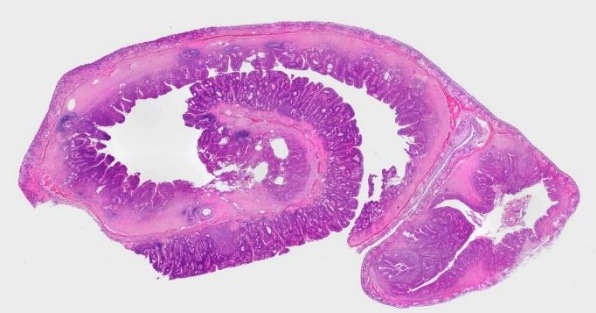
Nasal mucosa, sheep. Gross section of fixed turbinate. The mucosa at the top is approximately normal size, demonstrating the marked proliferation of the mucosa lining the scrolls (Photo courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu/)

tissues. There is dense fibrous tissue expanding the submucosa (fibrosis).

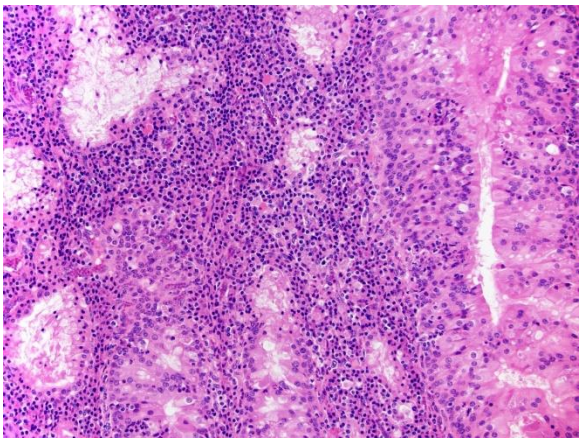
Contributor's Morphologic Diagnosis: Nasal cavity – Rhinitis, proliferative, lymphoplasmacytic and pyogranulomatous, diffuse, marked, chronic with bacteria.

Contributor's Comment: Chronic proliferative rhinitis has been reported in sheep from the United States, Spain, and most recently Switzerland.[1,2,5] Affected breeds in published reports include Columbian, Dorset, Aragonasa, and Texel sheep. Clinical signs and associated lesions manifest n animals older than 1 year of age, and most published cases are between 4 and 7 years of age. The earliest published report was associated with *S. enterica* spp. *Arizonae* (serotype III).[1] Other reports have identified *Salmonella diarizonae* (serotype IIIb: 61:k:1, 5,(7)) as the associated organism.[2,5] This latter serotype is commonly isolated from sheep in several countries and is considered host-adapted.[3]

In a recent survey of US sheep flocks by the USDA, 66.4% of flocks were culture-positive for *Salmonella* spp. and 94.6% of the isolates were identified as serotype IIIb61:k:1, 5,(7)[4] The intestine and tonsils are commonly colonized without any clinical signs or pathologic findings. There are few sporadic reports of associated diarrhea in lambs, abortions, and a report of epididymo-orchitis in a ram.[3] The bacterium can be detected in fecal swabs of healthy animals and is likely maintained in sheep largely due to fecal-oral transmission and colonization.[3] The bacterium can also be detected in nasal swabs from animals with chronic proliferative rhinitis making this another possible source of infection for other animals in a given flock.[5]



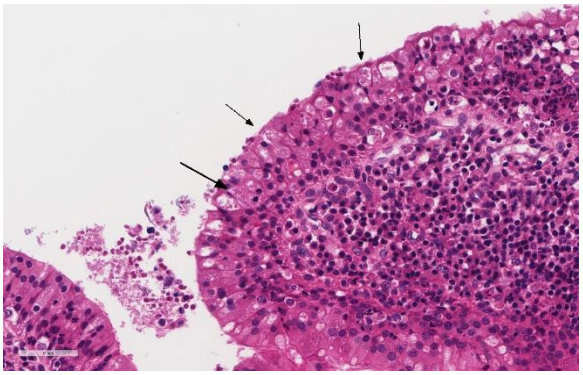
Nasal mucosa, sheep. Histologic section of the sample in Fig 2. (Photo courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu/) (HE, 7X)



Nasal mucosa, sheep. The lamina propria is expanded by a dense, primarily plasmacytic infiltrated. Overlying mucosa is markedly hyperplastic and glands are mildly degenerate and filled with sloughed cells and mucin. (Photo courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu/) (HE, 200X)

In all case reports which included clinical findings, nasal discharge, respiratory distress, and dyspnea of increasing severity over weeks to years were reported.[1,2,5] Depending on severity and chronicity, "striking mouth-breathing" and loss of body condition may also occur.[3] This may be fatal unto itself or lead to euthanasia.

The gross and microscopic appearance of this case is consistent with all published cases. The most prominent light microscopic features are the polypoid projections formed



Nasal mucosa, sheep. Mucosal epithelial cells are multifocally expanded by intracytoplasmic aggregates of bacilli (arrows). (HE, 315X)

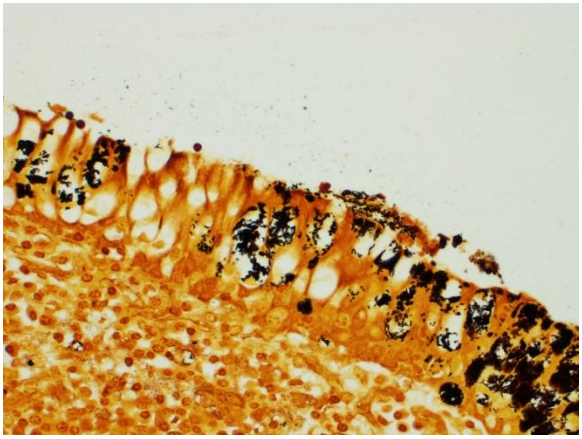
by hyperplastic epithelium and the infiltrate within the lamina propria and submucosa composed of large numbers of plasma cells, lymphocytes, neutrophils, and macrophages. Particularly prominent in this case were abundant, poorly visualized intracytoplasmic bacilli/coccobacilli within epithelial cells. Additional extracellular bacteria are evident with the aid of IHC. The factors which lead to this striking lesion in association with this common bacterium remain unknown. One recent study attempted to preproduce the disease in a controlled study with limited success.[3] Intranasal inoculation of bacteria led to elevated IFN-gamma over several months and bacteria were maintained in the respiratory tract for at least one year; however, the proliferative lesion was not reproduced. Upper respiratory tract diseases in sheep are most often seen related to infection by larvae of the bot fly *Oestrus ovis* (nasal myiasis; osteosis), leading to mild eosinophilic, catarrhal rhinitis. Enzootic nasal tumor, caused by enzootic nasal tumor virus -1 and -2 (oncogenic betaretrovirus) is another clinical differential, but is likely to cause a focal mass lesion and have a strikingly different histology appearance. Other sporadically occurring nasal tumors or space occupying lesion (e.g. lymphoma, non-viral adenocarcinoma and fungal granuloma) may also have similar clinical signs or gross lesions. Lastly viral pneumonias (e.g. respiratory syncytial virus, ovine adenovirus, parainfluenza 3) may present clinically as primarily upper respiratory signs.)

Contributing Institution:
The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu

JPC Diagnosis: Nasal mucosa: Rhinitis, proliferative**,** and lymphoplasmacytic, chronic, severe, with multifocal mucosal erosion and numerous intracytoplasmic bacilli.

JPC Comment: The contributor does an excellent and thorough writeup on the current knowledge of this uncommon but very characteristic lesion associated with *Salmonella* in sheep. This entity has made one previous appearance in the Wednesday Slide Conference (and must have made quite the impression during the AFIP residency of the moderator, who chose this counter-intuitive and unique lesion for a conference 29 years later.)

According to one report, there is concern over zoonotic potential for this bacterium within



Nasal mucosa, sheep. A Warthin-Starry stain demonstrates the large numbers of intracytoplasmic bacilli. (WS 4.0, 200X)

this particular presentation, although there is no evidence demonstrating actual occurrence. This particular serovar is most commonly seen following transmission from infected snakes with vertebral osteomyelitis and oophoritis.[3]

Nasal mucosa, sheep. Immunostaining for Salmonella sp. antigen demonstrates widespread immunopositivity within the mucosal epithelium of the submitted section (Photo courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu/) (anti-Samonella spp., 200X)

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CASE IV: M18-02265 (JPC 4118633).

Signalment: 3 year 8 month-old, female Friesian, bovine (*Bos taurus*)

History: On a farm in the Hunter region of NSW Australia, 2/40 adult cows died suddenly. Three days prior to death, the herd had been moved onto a Kikuyu (*Pennisetum clandestinum*) paddock. It was also possible that the herd had not had access to water for 24h.

Gross Pathology: Necropsy was performed on 2 animals. Gross findings in the necropsied animals included severe dehydration and abundant liquid content of the rumen. Ruminal papillae tips had multifocal pale discoloration. Mucosa from reticulum and omasum presented with multifocal red areas. The cows were pregnant with fetuses aged at approximately 7-8 months gestation

Laboratory results: Results of serum biochemistry analysis on an animal from the same herd were consistent with the gross finding of severe dehydration (decreased renal perfusion resulting in mild elevations in Urea/Creatinine/Phosphate and Protein/Albumin/Globulin) and muscle damage possibly related to being recumbent prior to death (mild elevations in creatinine and AST). Serum D-lactate levels were at the top of the normal range

			Reference Range
GGT	27		0-35 U/L
GLDH	20		0-30 U/L
AST	181	H	0-120 U/L
BIL	3.0		0.0-24.0 umol/L
CK	6752	H	0-300 U/L
UREA	17.5	H	2.1-10.7 mmol/L
CREAT	492	H	0-186 umol/L
PHOS	3.28	H	0.80-2.80 mmol/L
URE/CREA	0.04		0.00-0.07
PROTEIN	128.5	H	60.0-85.0 g/L
ALBUMIN	49.6	H	25.0-38.0 g/L
GLOB	78.9	H	30-45.0 g/L
ALB/GLOB	0.6	L	0.7-1.1
BHB	0.80		0.00-0.80 mmol/L
CA	2.62		2.00-2.75 mmol/L
MG	2.02	H	0.74-1.44 mmol/L
D-LACT	0.5		0.0-0.5 mmol/L

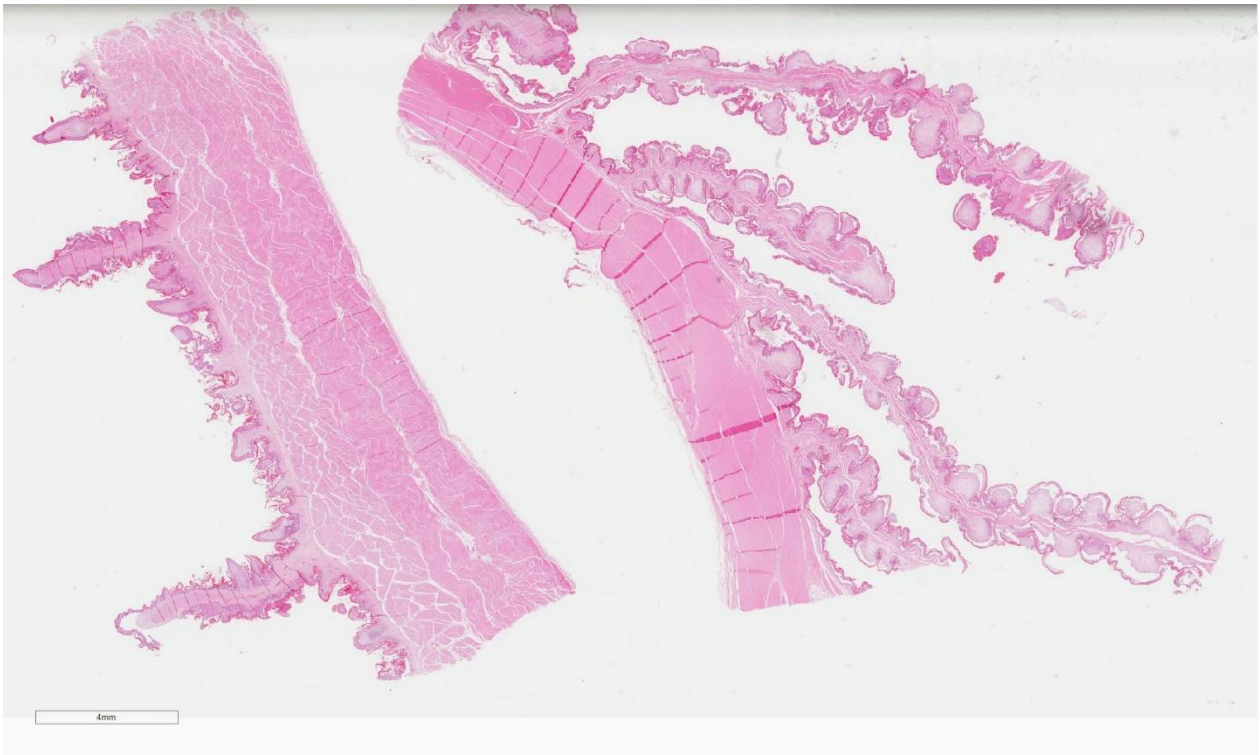
A Total Alfatoxins qualitative strip test on a sample of the kikuyu was negative (<4ppb cut off)

Microscopic Description: Omasum: There is widespread disruption of epithelial tissue architecture with multifocal to coalescing aggregates of degenerate neutrophils, epithelial cells with eosinophilic cytoplasm and shrunken, pyknotic or karrhorhectic nuclei admixed with cellular debris (micropustules). There is diffuse separation of omasal mucosa from underlying tissue (interpreted to be partially due to artifact). The omasal lumen is filled with fragments of refractile plant material mixed with myriad of small basophilic cocci. The submucosa is diffusely, mild to moderate expanded with clear areas (edema), with multifocal mild to moderate coalescing infiltrates of neutrophils, lymphocytes and plasma cells with occasional macrophages.
Reticulum: There is multifocal to coalescing disruption of epithelial tissue architecture with multifocal to coalescing aggregates of degenerate neutrophils, epithelial cells with eosinophilic cytoplasm and shrunken, pyknotic or karrhorhectic nuclei (interpreted as necrotic epithelial cells) (micropustules) admixed with clear spaces (edema) and cellular debris. Subepidermal vessels are diffusely congested and interstitial fibroblasts and myofibers are multifocal separated out by increased amounts of clear space.

Contributor's Morphologic Diagnosis: Omasum: Omasitis, necrosuppurative, diffuse, moderate to severe with intracorneal pustules, Friesian, bovine (*Bos taurus*)

Reticulum: Reticulitis, necrosuppurative, multifocal to coalescing, moderate with intracorneal pustules, Friesian, bovine (*Bos taurus*)

Contributor's Comment: Kikuyu grass (*Pennisetum clandestinum)* is a perennial tropical pasture species commonly found on coastal regions of Australia growing between spring and autumn.[2] Poisoning occurs after ingestion of kikuyu after a period of rapid



Ox, reticulum and omasum: At low magnification, the mucosa is multifocally lifted off of the underying mildly edematous submucosa. (HE, 5X)

growth in autumn following a period of summer drought. From a collation of outbreaks across 40 farms, between 13.6% and 64.2% cattle were affected of which between 16.7% and 95.6% died.[2] In an outbreak, signs of toxicity typically present on average 3 days (1-8 day range) post first grazing of kikuyu, with some cases progressing quickly to death over a subsequent week long period.[2] Clinical signs consist of drooling, dehydration, abdominal pain, sham drinking, depressed mentation, incoordination and recumbency. Other signs may include distended abdomen, rumen stasis, elevated temperature and both cardiac and respiratory distress.[2,3] A history of grazing kikuyu and a post mortem finding of a distended rumen containing a sloppy mix of pasture and fluid are highly suggestive of kikuyu poisoning. Additional supportive evidence from a post mortem includes hyperaemic mucosa of the forestomaches and abomasum, empty small intestine, dry contents in the large intestine and cardiac haemorrhages.[2] The most consistently reported histopathological lesion is segmental necrotising inflammation within the epithelium of the forestomach mucosa,[2-4] most frequently the omasum.[2] Cases of longer duration of exposure show mucosal inflammation regresses and epithelial repair occurring.[2]

There are multiple theories towards the toxin production that causes kikuyu poisoning. While produced seasonally, it is unknown if it is produced spontaneously by the plant, in response to a stimulus such as a pathogen or by a pathogen itself. Despite no reported cases of nitrate poisoning from kikuyu, one suggestion is the accumulation of nitrogenous compounds may be associated with toxin production.[2] Another is the association of armyworms and kikuyu in

some but not all cases[1]. Oral ingestion of fungi cultures and mycotoxins has produced similar kikuyu-like poisoning conditions in cattle, however isolation of these fungi and/or mycotoxins from kikuyu plants and soil associated with poisonings is not consistent.[1,2,6]

Other diseases with a similar presentation to kikuyu poisoning include metabolic disease such as hypocalcaemia and hypo-magnesemia, infectious diseases such as listeriosis and *Histophilus somni* meningoencephalitis, or other poisonings such as lead, salt or nitrate poisoning. Ruminal acidosis causes similar histological lesions, but is usually restricted to the rumen.

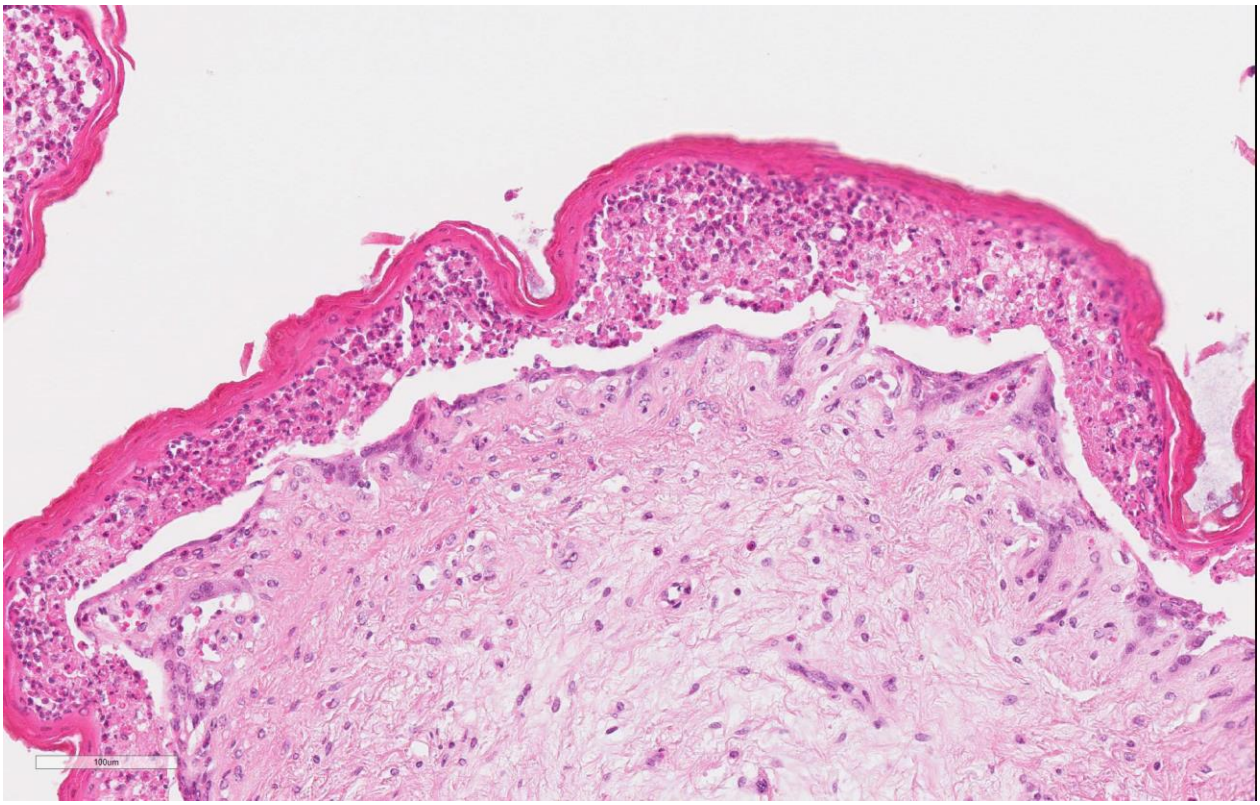
Contributing Institution: Elizabeth Macarthur Agricultural Institute, NSW, DPI https://www.dpi.nsw.gov.au

JPC Diagnosis: 1. Omasum: Omasitis, necrotizing, multifocal to coalescing, with numerous intracorneal pustules.

2. Reticulum: Reticulitis, necrotizing, multifocal to coalescing, with numerous intracorneal pustules.

JPC Comment: Kikuyu grass (*Pennisetum clandestinum*) is a drought-tolerant grass native to the region of Kenya that is home to the Kikuyu people. It has been adapted to be a lawn grass in dry regions of New Zealand, South Africa, and the Southwestern US (California). It has become popular on golf courses due to its dense nature and propensity to create challenging rough. It has been termed an invasive species in several countries due to its ability to overgrow other plant life, preventing other plants from sprouting and producing herbicidal toxins that compete with other plants.[5]

Toxicity is sporadic, with many animals grazing it with no ill effects. Outbreaks are



Ox, omasum. Coalescing intracorneal pustules often form clefts within the mucosa. (HE, 246X)

brief, geographically restricted, and lethal. A number of strains (with such names as "Whittet", "Crofts", and "Noonan") of kikuyu grass have been developed but do not appear to differ significantly in potential toxicity.[2]

The contributor mentions the "sloppy mix" of pasture and fluid in the rumen. This appears to be the result of a number of contributing factors, to include excess salivary production, ruminal stasis and a net outflow of fluid from the ruminal mucosa into the ruminal lumen. Animals will excessive amounts of ruminal fluid may "sham drink" (placing the head in or near water or playing in a water source without actual drinking) and die of dehydration due to third spacing of water into the rumen.[2]

The microscopic lesion demonstrated in this case is consistent with that described in cases of kikuyu poisoning – necrosis of the superficial layers of the ruminal, reticular, and omasal mucosa (stratum lucidum, granulosum, and spinosum) with profound infiltatin by neutrophils which lifts it off of the underlying stratum basale. Degenerative changes in the myocardium and kidney have been infrequently reported in association with this condition.[2]

The identity of the toxin in kikuyu grass is yet to be elucidated and is the subject of many theories regarding its identity and seasonality. The forestomach lesion is reminiscent of that seen with ingestion of *Baccharis* sp. plants by ruminants (WSC Conference 2012-2013, Conference 4, Case 2), which is the result of accumulation of fungus-produced trichothecene toxins. A kikuyu-lke condition has been reproduced in ruminants via feeding of fungal cultures from two pasture fungi, *Myrothecium* sp. and *Phoma* sp. which produce roridin and verrucarin toxins, respectively. Nitrates have often been incriminated in outbreaks of kikuyu grass, which accumulated nitrates during their reproductive period within their stems. Leaf damage by various insects have been theorized to increase the consumption of kikuyu stems in some outbreaks, or overgrazing may increase stem consumption. In most reported cases of kikuyu poisoning, nitrate levels were within normal limits in affected animals.[2]

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