Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 15

20 January, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: Case 2 N-169/20 (4153943-00)

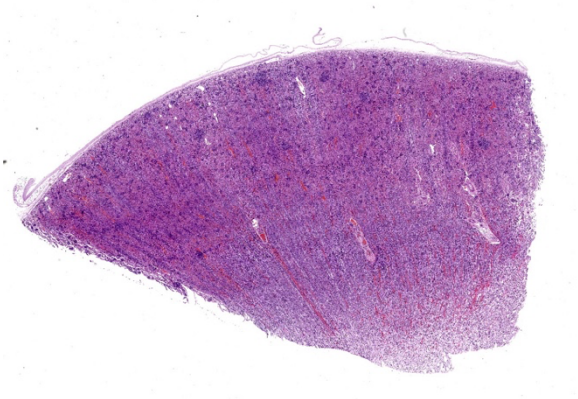
Signalment:
1-day old foal, male, Pure Spanish Breed, Equine (*Equus ferus caballus)*.

History:
The physical examination reveals hyperthermia and weakness. Supporting treatment consisting of IV fluid therapy, antibiotic (cefquinoma) and NSAID (flunixin). The animal died 12 hours later.

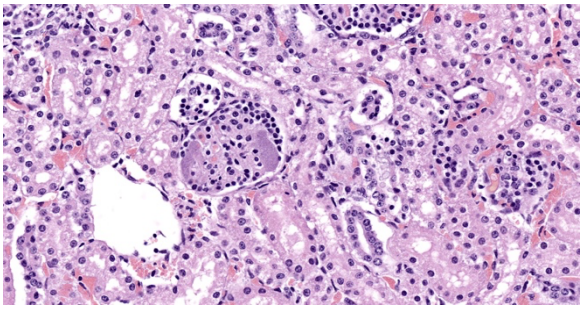
Gross Pathology:
The kidneys were swollen, diffusely congested and up to 10% of the renal cortex had multiple, 1-2 mm diameter, well demarcated, round, white foci (embolic nephritis).

Laboratory results:
None submitted

Microscopic description:
Kidney: Multifocally, up to 20% of the renal cortex is affected by an inflammatory and necrotizing process. Diffusely, expanding and effacing the glomeruli and peripheral tissue, there are abundant viable and degenerated neutrophils, moderate number of macrophages, few lymphocytes and plasma cells, admixed with abundant eosinophilic cellular debris with pyknosis and karyolysis with loss of cellular outlines (lytic necrosis), moderate amount of fibrin, hemorrhage, edema and small to medium extracellular basophilic colonies of 1-2µm coccobacilli (microabscesses). Adjacent tubular epithelium variably presents one of the following changes: shrunken hypereosinophilic cytoplasm with pyknosis (coagulative necrosis) or swollen pale eosinophilic vacuolated cytoplasm (tubular degeneration). Diffusely, the interstitium shows moderate congestion. Remaining tubules present minimal tubular degeneration with few intraluminal, pale round eosinophilic material (protein cast).



Kidney, foal. A section of kidney with cortex and outer medulla is submitted for examination. Lesions are not apparent at subgross examination (HE, 5X)



Kidney, foal. Fetal glomeruli are prominent in the section. Multifocally and randomly, there are emboli of large numbers of 1-2um coccobacilli occluding glomerular capillaries (arrows), and affected tufts are segmentally expanded by low numbers of necrotic neutrophils, fibrin, edema, and cellular debris. (HE, 400X).

Contributor's morphologic diagnosis:

1. Kidney: Multifocal suppurative glomerulonephritis diffuse, global, subacute, moderate with intralesional small colonies of coccobacilli.
2. Kidney: Tubular degeneration and necrosis, acute, diffuse, mild.

Contributor's comment:

Actinobacillus equuli, a Gram-negative pleomorphic rod, is the causative agent of several diseases in horses. The most serious is an acute, usually fatal septicemia of newborn foals known as: "sleepy foal disease",[4] which causes lesions at several organs, including the kidneys, joints, lungs and intestine. The most likely source of infection for the neonate is the mare, where the organism is present in the normal oral flora.[4]

A. equuli affects more the newborn foals, in adult horses are less common and generally more localized, causing acute and chronic peritonitis.[11]

Rarely, *A. equuli* has been reported to be an opportunistic pathogen of pigs, with these infections typically associated with abortion, septicemia, and polyarthritis.[11]

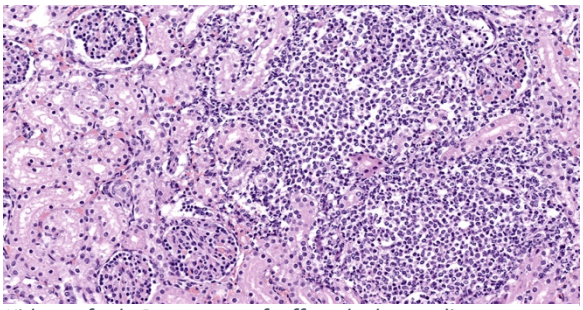
Fecal contamination or extension from oral mucous membranes is the method of inoculation, is acquired in utero during parturition, or shortly after birth as an umbilical infection, the last one is the most common route of infection in foals, resulting in septicemias.[1,5]

Two subspecies of *Actinobacillus equuli*, subsp *equuli* and *haemolyticus* are normal inhabits of mucous membranes of the alimentary tract. Subspecies are based on the repeats-in-toxin (RTX), toxin genotype (*Aqx-equuli* toxin).[1,7] The higher isolation rates of *A. equuli* subsp *equuli* over *A. equuli* subsp *haemolyticus* from septicemic cases indicates that other virulence factors play a major role in pathogenesis. Toxins belonging to RTX family are produced by some species of *Actinobacillus*, and they exert hemolytic, cytotoxic activity and host specificity.[7,8,12]

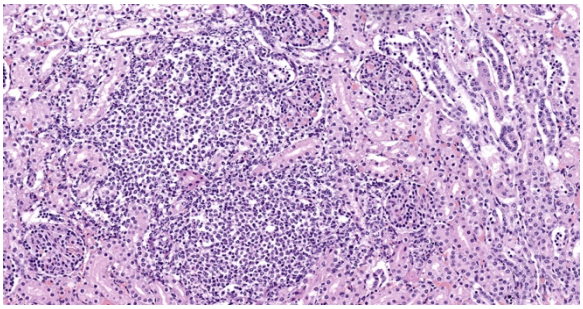
The foal is born without anti-Aqx antibodies but acquires them by passive transfer from the mare's colostrum within an hour of ingesting it. The complete failure of passive transfer caused by the delay in the colostrum supply, or by a poor-quality colostrum predisposes foals to infection and sepsis.[2,4]

A. equuli is of clinical importance, since it can cause disease in horses and is found in infected wounds of humans bitten by horses. Hemolytic strains of *A equuli* are also isolated from tracheal washes, indicating a preference for the respiratory tract. These strains are opportunistic pathogens and can cause respiratory infections, septicemia, metritis, mastitis, arthritis, endocarditis, meningitis and peritonitis. Non-hemolytic strains is well known to cause the clinical condition: 'sleepy foal disease.'[4]

A. equuli is a well-known cause of embolic nephritis in young horses. At gross examination small abscesses occur in a variety of organs, including the liver, adrenal gland, joints, and the



Kidney, foal. Remnants of effaced glomeruli are present within expanding foci of septic and neutrophilic inflammation. (HE, 255X)



Kidney, foal. Foci of suppurative inflammation are scattered throughout the cortex, expanding the cortex and effacing glomeruli and tubules. (HE, 250X)

kidney.[1] Microscopically, glomerular capillaries and to a lesser extent interlobular arterioles contain microabscesses. If the animal survives the infiltrate can persist as focal residual abscesses or coalescing scar.[1]

Embolic nephritis also occurs commonly in the bacteremia of pigs infected with *Erysipelothrix rhusiopathiae*, sheep and goats infected with *Corynebacterium pseudotuberculosis, Trueperella pyogenes* is the most common isolate in cattle, but *Staphylococcus aureus, Mannheimia haemolytica* and *Streptococcus bovis* were also present.[1]

Contributing Institution:
Universidad de Zaragoza
Departamento de Patología Animal
https://patologiaanimal.unizar.es

JPC diagnosis:
Kidney: Nephritis, suppurative, embolic, with mild fibrinosuppurative glomerulitis and rare large colonies of bacilli.

JPC comment:
The contributor provides a concise summary of this disease. In addition to the described diseases in horses, *Actinobacillus equuli* ssp. *equuli* is one of the most commonly isolated agents causing osteomyelitis in foals, while *Actinobacillus equuli* ssp. *haemolytica* is a common cause of bronchopneumonia and pleuritis in horses.[6]

A number of species within the *Actinobacillus* genus are closely related, and *A. equuli* is difficult to differentiate from *A. suis* using even 16S rRNA sequencing. Recent whole genome sequencing of the *Actinobacillus* genus phylogenetic tree shows that a number of species capable of causing invasive disease cluster closely by surface antigen type, and include *A. suis*, *A. ureae*, *A. equuli equuli*, and *A. capsulatus*.[3]

As stated previously, *A. equuli* also occasionally affects pigs and piglets. Recently, an outbreak of *A. equuli* caused swollen joints and moderate to severe lameness in 6-8-hour old piglets. They experienced lethargy and a subset became non-ambulatory. The primary findings in the affected piglets included purulent polyarthritis, tendovaginitis, and purulent inflammation in the brain and kidneys of one animal.[8]

During the conference, the moderator emphasized how glomerular morphology in this case allows for some age determination of the animal. The glomerular visceral epithelial cells are plump podocyte precursor cells, consistent with fetal glomerular morphology of a young animal. It was also emphasized that this agent, as well as other possible bacterial agents, will often be found in the peritubular capillaries.

The moderator reminded all participants that the vessels in the kidney are arteries, with the term "arteriole" being reserved for the afferent and efferent vessels immediately arriving and departing the glomerulus, respectively. Arterioles lack the internal elastic lamina, allowing for direct and immediate control of lumen diameter and blood pressure.

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CASE 2: Case 1 GR (4153953-00)

Signalment:
Age: 35-42 days; Gender: 2 females and 1 male; Breed: Yorkshire; Scientific name: *Sus Scrofa Domesticus*; Species: Domestic Pig

History:
The present cases (three piglets) were the offspring of a sow that had been born in a litter where several littermates had died with renal lesions. This sow was experimentally artificially inseminated with semen from a boar that previously had produced litters where several piglets had died with renal lesions before weaning.

Gross Pathology:
The carcasses of these piglets were conspicuously anemic. The myocardium of the left heart ventricle was thickened due to hypertrophy. The stomach contained some coagulated blood, and erosions and hemorrhagic ulcerations were observed in the cutaneous mucosal lining of the *pars oesophagea*.

In the retroperitoneal abdomen there was a well-developed perirenal edema. The renal capsule could be easily removed. The kidneys were enlarged and somewhat pale yellow-brownish discolored with a finely granular surface containing numerous diffusely distributed cortical petechial hemorrhages. The cortical surfaces had several linear shallow indentations radiating from the renal hilum out to the renal lateral border. At outside inspection the kidneys appeared somewhat "collapsed" flattened at the hilum. After sagittal section of the kidneys the renal papillae on the cut surface appeared atrophic giving the impression of dilated calyces mimicking hydronephrotic change.

Laboratory results:
Median serum urea concentration: 44.9 mmol/L; Median serum creatinine concentration: 848 µmol/L; Microbiology (kidney, spleen and blood): No bacterial growth; Median plasma C3 concentration: 4,5 % i.e. hypocomplementemic; Median plasma Terminal Complement Complex (TCC; "MAC") concentration 13,6 Arbitrary Units (AU)/mL as compared to median plasma TCC concentration in healthy age-matched piglets of 1.8 AU/mL.

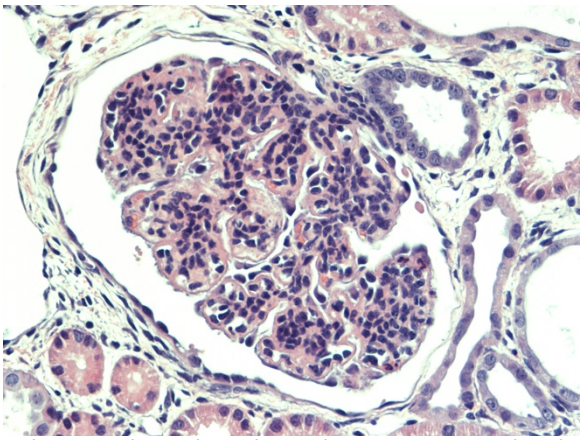
*Kidney, piglet. Capsular and cut surface of kidney. The capsular surface has a dry pebbled appearance, and there is mild hydronephrosis. (Photo courtesy of: Faculty of Veterinary Medicine, Oslo, Norway, **www.nmbu.no**)*

Microscopic description:
Renal tissue: All glomeruli had a principal glomerular lesion that was characterized by a combination of diffuse glomerular capillary wall thickening and pronounced mesangial cell hyperplasia. This glomerular lesion gave the glomeruli an accentuated lobulation with loss of glomerular capillary patency. Several glomeruli revealed more exudative changes with evidence of glomerular capillary infiltration of neutrophilic granulocytes (PMN-N) and fibrin exudation with a variable number of PMN-Ns to the urinary spaces (within the Bowman capsules) with a varying degree of fibrinocellular crescent formation, corresponding to a rapidly progressive glomerulonephritis (RPGN).

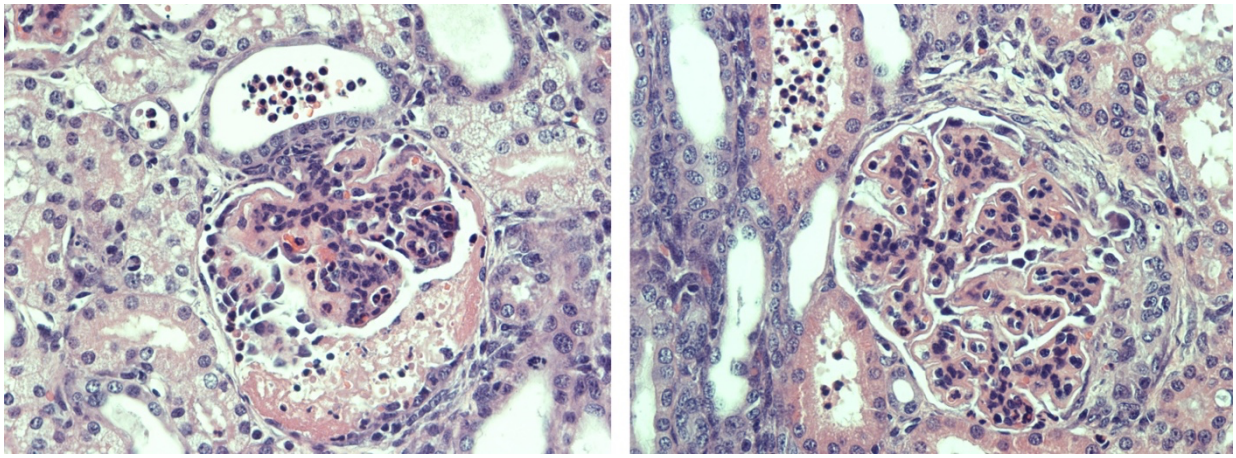
The proximal tubular epithelium revealed degenerative changes with conspicuous evidence of massive hyaline droplet degeneration. A vast number of distal tubular segments and collecting ducts contained cylinders of proteinaceous fluid with a variable content of PMN-N´s. The renal cortical interstitium was moderately expanded by collagen, and the medullary interstitium was conspicuously expanded by fibrocytes/fibroblasts. In the papillary medullary tissue, there was a scarcity of collecting ducts and other tubular elements such as the loops of Henley.

Immunohistochemistry / Immunofluorescence
Immunohistochemistry and immunofluorescence studies of the glomeruli were consistently negative for immune-complex depositions. Indirect immunofluorescence on frozen kidney sections for glomerular porcine C3 (pAb SwineC3) was strongly positive along the glomerular capillary walls and in the expanded mesangium, and mAb HumanTCC ("MAC") was consistently strongly positive mainly along the thickened glomerular capillary walls. Double labeling showed colocalized staining (yellow) along capillary walls, while C3 deposition dominated in the mesangium. Green: FITC (fluorescein isothiocyanate. Red: TRITC (tetramethylrhodamine).

Transmission electron microscopy (TEM):
The ultrastructural appearance of the GBM revealed a conspicuously thickened *lamina densa* consisting of a homogenous electron dense osmiophilic material. The thickness of the GBMs were measured to 1000-1250 nm as compared to the normal GBM thickness of about 150 – 200 nm. The glomerular visceral epithelium (the



*Kidney, piglet. The glomerulus is segmented. The mesangium is markedly hypercellular and glomerular capillary walls are diffusely thickened. (HE, 400X). (Photo courtesy of: Faculty of Veterinary Medicine, Oslo, Norway, **www.nmbu.no**)*



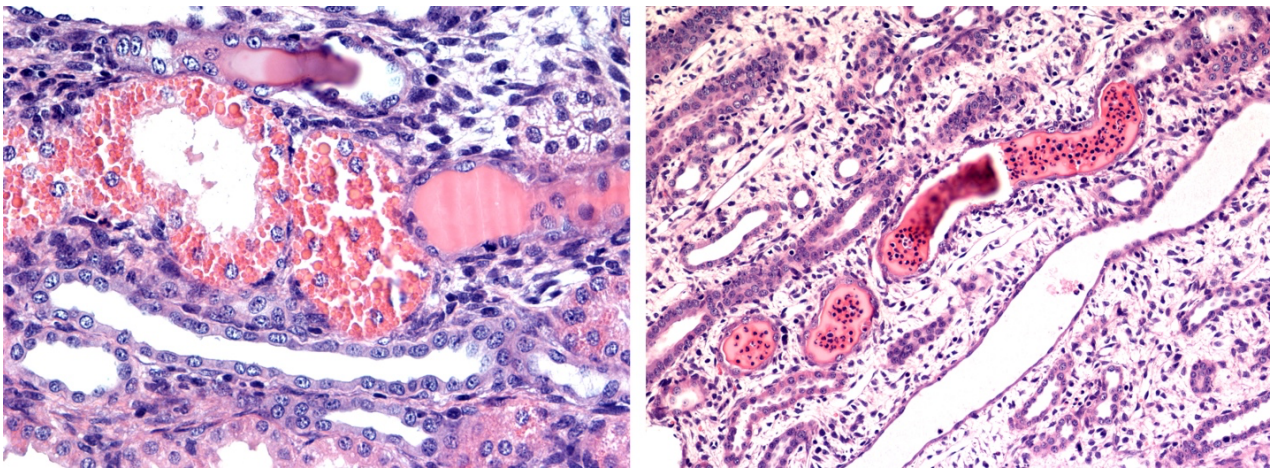
*Kidney, piglet. (Left) The uriniferous space is expanded by abundant fibrin with small amounts of cellular debris. (Right) Glomerular synechiation, organization of fibrin exudate, segmental fibrosis and hyperplasia of the parietal epithelium (crescent formation) (HE, 400X). (Photo courtesy of: Faculty of Veterinary Medicine, Oslo, Norway, **www.nmbu.no**)*

podocytes) revealed general loss of foot processes and showed a general attenuation along the outside of the thickened electron dense GBM.

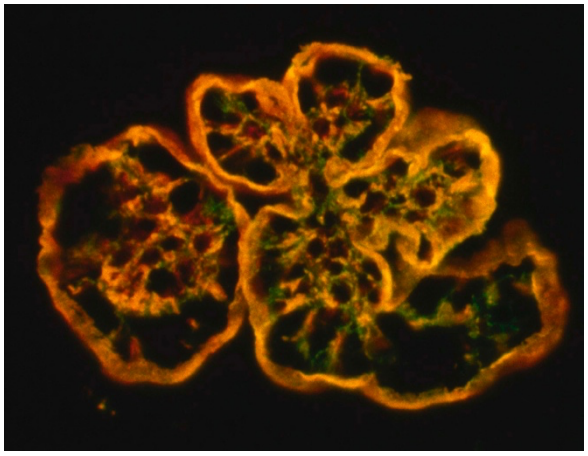
Contributor's morphologic diagnosis:
Porcine Membranoproliferative Glomerulonephritis type II; Porcine C3 glomerulopathy, aka (Porcine) Dense Deposit Disease (DDD)

Contributor's comment:
Glomerular diseases represent important causes of renal failure because they cause loss of glomerular function and impair blood flow within peritubular capillaries downstream of the glomeruli, affecting the entire nephrons. Pro-inflammatory cytokines, chemokines, and growth factors produced by glomerular inflammatory reactions and transported further with the ultra-filtrate and the peritubular capillary blood may also affect and cause activation of the epithelium along the entire nephron as well as the renal interstitium, causing tubular degeneration and atrophy, and interstitial fibrosis. This adds to the devastating effects of increased protein-concentration in the pre-urine.

Glomerular diseases are termed primary when the kidneys are the only or predominant organs affected as opposed to secondary glomerular disorders caused by systemic diseases, e.g. systemic lupus erythematosus (SLE), metabolic



*Kidney, piglet. (Left) The cytoplasm of degenerate epithelial cells contains numerous brightly eosinophilic cytoplasmic inclusions (hyalinosis). (Right) Tubular lumina contain protein and occasionally neutrophils; the interstitium is edematous. (HE, 400X) (Photo courtesy of: Faculty of Veterinary Medicine, Oslo, Norway, **www.nmbu.no**)*



*Kidney, piglet. Double-labeled immunofluorescence with antibodies against C3 and human terminal complement complex (TCC). (Photo courtesy of: Faculty of Veterinary Medicine, Oslo, Norway, **www.nmbu.no**)*

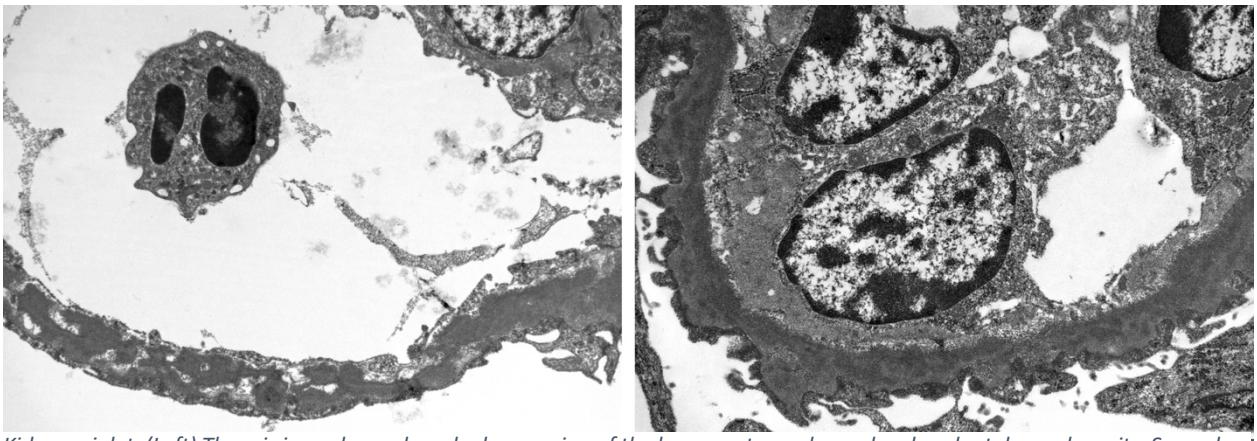
diseases, hypertension, or amyloidosis. Thus, glomerular diseases may be caused by both non-inflammatory and inflammatory reactions. However, both the clinical manifestations and glomerular histologic changes in primary and secondary forms can be similar. In cases of glomerular disease without any obvious inflammatory component, these conditions are termed glomerulopathies as opposed to more obvious inflammatory diseases which may be termed glomerulonephritides.

Primary glomerulopathies (-nephritides) are most often caused by immune-complex depositions within in the glomeruli, either as a result of deposition of circulating immune-complexes or immune-complexes caused by antibodies reacting *in situ* within the glomeruli, either by binding to insoluble fixed (intrinsic) glomerular antigens or to molecules planted within the glomerulus.

The antigens responsible for the immune-complex depositions are frequently antigens of infectious microorganisms but may also be self-antigens in the case of auto-immune reactions as e.g. in SLE or in neoplasia.

Primary glomerulopathies may also be caused by hereditary disease conditions as e.g. in hereditary complement factor 3 deficiency, Alport disease ("hereditary nephritis"; mutations of collagen IV), or collagen type 3 glomerulopathy caused by a hitherto unknown mutation.

The morphologic glomerular lesions in animal immune-mediated glomerulonephritides may vary, but hypercellularity (proliferation of resident glomerular cells, leukocytic infiltration, glomerular crescent formation), GBM thickening observed as a uniform, diffuse global thickening of the glomerular capillary walls with light-microscopy, and hyalinosis and sclerosis may be observed. Both membranous glomerulopathy and membranoproliferative glomerulonephritis (MPGN) are frequently diagnosed in animal species.



*Kidney, piglet. (Left) There is irregular and marked expansion of the basement membrane by abundant dense deposits. Secondary and primary foot process are absent. A neutrophil is present within the capillary lumen. (Right) A second cross section of capillary demonstrates similar basement membrane expansion and two hypertrophic endothelial nuclei. There is effacement of secondary foot processes. (9300X) (Photo courtesy of: Faculty of Veterinary Medicine, Oslo, Norway, **www.nmbu.no**)*

The MPGN occurring in animals are lesions similar to the human MPGN type I characterized by occurrence of sub-endothelial and para-mesangial glomerular immune-complexes evident as multifocal glomerular deposits of electron-dense material interposed between the glomerular basement membrane and the glomerular capillary endothelium and in the para-mesangial regions as observed with transmission electron microscopy (TEM).[1,2] These immune complexes trigger classical pathway complement activation and thus cause the inflammatory reaction.

Until 1993 another type of MPGN described in humans, MPGN type II with intramembranous dense deposits - (Dense Deposit Disease; DDD), had never been observed in any animal species, when it was discovered as the cause of disease and death in Norwegian Yorkshire piglets.[6] Since then, this type of glomerulonephritis has by consensus been re-named C3 glomerulopathy, denoting a glomerular lesion characterized by C3-accumulation with absent or scanty immunoglobulin deposition.[10,12]

This disease condition in the Norwegian Yorkshire piglets was proven to be an autosomal recessive transmitted disease caused by an inherited deficiency of the fluid phase complement regulatory protein complement factor H (FH). The FH deficiency caused spontaneous unrestricted alternative complement activation when plasma was exposed to the glomerular basement membrane that is not protected by cell membrane-bound other complement inhibitory proteins. This porcine glomerular disease model was the first demonstration of the relationship between development of glomerular DDD and unrestricted glomerular alternative complement activation.[5]

In the project studying this inherited disease it was produced in all 317 piglets in a total of 28 litters, using artificial insemination of both frozen and fresh semen to sows associated to the disease condition by being littermates of piglets that had died from this disease. During this study the glomerulonephritic piglets were detected by having abnormally low plasma concentrations of Complement factor 3 (C3) and abnormally high plasma concentrations of fluid phase TCC ("MAC"). 88 (27.8 %) of the produced piglets were diagnosed as FH-deficient and developed all - without exceptions – lethal Porcine Dense Deposit Disease (previously named Porcine Membranoproliferative Glomerulonephritis type II).

As heterozygote carriers of the genetic mutations revealed about half-normal plasma concentration of C3, we were able to detect all carriers within the Norwegian breed herds for the Norwegian Yorkshire breed. All diagnosed carriers were removed from the breeding program, and thus this disease was eradicated from the Norwegian Yorkshire pig population and has not been observed to re-emerge.[3-9]

Contributing Institution:
Faculty of Veterinary Medicine
Oslo, NORWAY
www.nmbu.no

JPC diagnosis:
Kidney: Glomerulonephritis, proliferative and crescentic, diffuse, severe with tubular degeneration, necrosis, and regeneration, protein and cellular casts, and edema.

JPC comment:
The contributor provides an excellent review of this disease while highlighting the different subtypes of membranoproliferative glomerulonephritis (MPGN). There has been reorganization of, and recently proposed categorizations within, human MPGN. Type I and III MPGN is still related to immune complex deposition and is stratified by polyclonal or monoclonal antibody deposition. However, now three entities are grouped within a new category called C3 glomerulopathy (C3 GP), all requiring dysfunction of the alternative pathway of complement activation: Dense Deposit Disease (DDD), C3 glomerulonephritis (C3 GN), and complement factor H-related protein 5 glomerulopathy (CFHR5 GP). In order to arrive at a diagnosis of one of these diseases, a combination of light microscopy, immunofluorescence/immunohistochemistry and TEM, laboratory complement findings, and

clinical history and data are required. Unfortunately, it is rare that the veterinary pathologist has access to all these data.[11]

The alternative pathway of complement activation relies on neither immune complexes nor bacterial sugars and is constantly in a moderate state of activation. Factor B binds to C3b and factor D cleaves the complex to form the active C3bBb that has C3 convertase activity. Regulatory mechanisms become important it a process that is constantly in action, and factors H and I play critical roles. Factor H inhibits the formation of C3 convertase, while factor I inhibits C3b by proteolysis. Deficiency of factor H results in severe secondary depletion of intact C3, while deficiency of factor I results in accumulation of C3b in plasma. As described by the contributor, Dense Deposit Disease in pigs is specifically the result of complete factor H deficiency. Murine models of this disease have successfully been created, with a targeted gene deletion of exon 3 of murine factor H gene within embryonic stem cells. The mouse model is a good representation of Dense Deposit Disease, across humans and pigs.[13]

Conference discussion centered briefly on the glomerular crescents in this section. When confronted with glomerular injury, pigs often make large, distinct glomerular crescents, composed of fibrin, neutrophils, sloughed parietal and visceral epithelial cells, and ultimately leading to scarring. The moderator also shared that in C3GN, the electron dense deposits seen in this disease have a multisegmented shape, with a resemblance to sausage links. Immune complex deposition results in more discrete aggregates of electron dense material.

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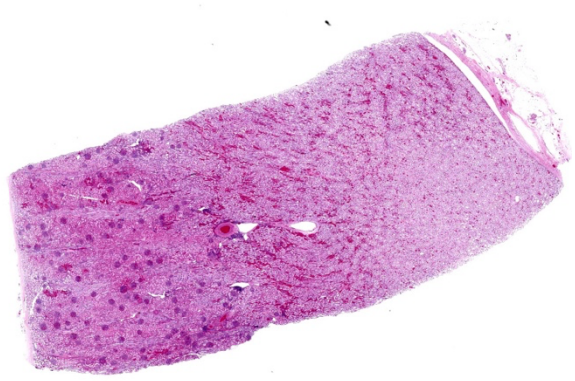
CASE 3: 18N2812 (4135943-00)

Signalment:
Adult, 20+ year-old Quarter Horse gelding, *Equus caballus*

History:
A 20+ year old Quarter horse gelding presented for a fever of unknown origin, anorexia and approximately 1-week history of nasal discharge and cough following multiple horses on the property confirmed to have influenza. Non-steroidal ant-inflammatories (NSAIDs) were not given prior to presentation. On abdominal ultrasound there was an abnormal echotexture and shape to the right kidney. Blood creatinine levels were also elevated (5.3 mg/dl; reference range: 0.9-2.0 mg/dl). Creatinine levels continued to rise in the face of fluid therapy. Due to poor prognosis, the owners elected for humane euthanasia.

Gross Pathology:
The pleural cavity contains approximately 1 liter of serosanguinous effusion. The lungs are mottled pale to dark pink. Approximately 25% of the cranioventral lung lobes are bilaterally firm and consolidated and sinks in formalin. The caudodorsal lung lobes are bilaterally soft to rubbery and floats in formalin. On cut section, the lung oozes clear, watery fluid.

There is approximately 1 liter of yellow to light brown, translucent, watery fluid present in the peritoneal cavity. Beneath the renal capsule, randomly, scattered over the entire surface of kidneys are hundreds of petechiae which range from 1 to 2 mm in diameter. On cut section the entire cortex of each kidney is similarly affected. Diffusely, the corticomedullary junctions are dark red. The renal pelvices, bilaterally, contains, pale tan, folds of undulating tissue, which is expanded by clear, watery to gelatinous, fluid. Segmentally, 6.5 cm of the right proximal ureter and 5.0 cm of left proximal ureter are transmurally red. The serosal and mucosal vasculature is prominent and congested at the apex of the bladder. Additionally, within this affected region of bladder mucosa there is a 0.5 cm in diameter purpura. The lumen of the bladder contains cloudy, dark yellow to brown urine.



Kidney, horse. A section of kidney, including cortex (left) and medulla (right) is submitted. (HE, 5X)

Laboratory results:
CBC:
Echinocytosis (many)
Left shift (bands; 108/ul)
Mild monocytosis (867/ul)
Mild basophilia (217/ul)
Fibrinogenemia (800 mg/dl)

Interpretation: *Inflammatory leukogram*

Chem:
Hypophosphatemia (1.3 mg/dl)
Azotemia: elevated BUN (36 mg/dl) and creatinine (5.3)
Hypoalbuminemia (2.1 g/dl)
Hyperglobulinemia (5.3 g/dl)
Low creatinine kinase (108 IU/L)
Hypobilirubinemia (<0.2 mg/dl direct)

Interpretation: *Hypoalbuminemia, considerations include loss (renal or GI) or decreased production (no support). Histopathology suggests glomerular disease and therefore renal loss. Azotemia may be pre-renal, but histopathology suggest renal is primary. Hypophosphatemia may be secondary to alkalosis, supported by respiratory disease observed on necropsy, but renal losses and GI malabsorption cannot be fully excluded. Recommend blood gas, urinalysis and fractional excretion of phosphorous.*

SAA:
Elevated serum amyloid A (SAA); (2930 ug/ml)

Interpretation: *Inflammation*

ERYTHROCYTE PARAMETERS	RESULT	EQ REF	UNITS	COMMENTS
Rbc	6.18	6.2-10.2	M/ul	
Hemoglobin	10.4	11.2-17.2	gm/dl	
Hematocrit	30.4	30-46	%	
Mcv	49.2	37-53	fl	
Mch	16.8	14-20	pgm	
Mchc	34.2	36-39	gm/dl	
Rdw	17.0	16-20	%	
RBC MORPHOLOGY	**RESULT**			
Anisocytosis	SLIGHT			
Echinocytes	MANY			
LEUKOCYTE PARAMETERS	**RESULT**	**EQ REF**	**UNITS**	**COMMENTS**
Wbc	10,840	5000-116000	/ul	
Bands	108	RARE	/ul	
Neutrophils	8,022	2600-6800	/ul	
Lymphocytes	1,518	1600-5800	/ul	FEW REACTIVE
Monocytes	867	0-500	/ul	
Eosinophils	108	0-200	/ul	
Basophils	217	0-100	/ul	
OTHER PARAMETERS	**RESULT**	**EQ REF**	**UNITS**	**COMMENTS**
Platelets (x10^3)	148,000	100-225	/ul	NO CLUMPS SEEN
Mpv	6.2	5.4-9.3	fl	
Plasma Protein	7.2	5.8-8.7	gm/dl	
Plasma Fibrinogen	800	100-400	mg/dl	
Protein:Fibrinogen	8			
Icterus				SLIGHT ICTERUS

Equine Chemistry Panel 2	Result	EQ Ref	Units	Comments
MAGNESIUM, IONIZED	0.70	0.47-0.70	MMOL/L	
ANION GAP	13	9-17	MMOL/L	
SODIUM	133	125-137	MMOL/L	
POTASSIUM	5.0	3.0-5.6	MMOL/L	
CHLORIDE	95	91-104	MMOL/L	
BICARBONATE	30	23-32	MMOL/L	
PHOSPHORUS	1.3	2.1-4.7	MG/DL	Results have been verified by repeat analysis.
CALCIUM	12.7	11.4-14.1	MG/DL	
BUN	36	12-27	MG/DL	
CREATININE	3.5	0.9-2.0	MG/DL	
GLUCOSE	107	50-107	MG/DL	
TOTAL PROTEIN	7.4	5.8-7.7	G/DL	
ALBUMIN	2.1	2.7-4.2	G/DL	
GLOBULIN	5.3	1.6-5.0	G/DL	
AST	171	168-494	IU/L	
CREATINE KINASE (CK)	108	119-287	IU/L	
ALKALINE PHOSPHATASE	138	86-285	IU/L	
GGT	11	8-22	IU/L	
TRIGLYCERIDES	26	2-41	MG/DL	
BILIRUBIN TOTAL	0.8	0.5-2.3	MG/DL	
BILIRUBIN DIRECT	<0.2	0.2-0.6	MG/DL	
BILIRUBIN INDIRECT	N/A	0.3-1.7	MG/DL	Unable to calculate due to low Direct Bilirubin
SDH-37	7	0-8	IU/L	
HEMOLYSIS INDEX	0	0-14		
ICTERIC INDEX	1	0-4		
LIPEMIC INDEX	6	0-<1		

Creatinine	Result	EQ Ref	Units	Comments
CREATININE	5.3	0.9-2.0	MG/DL	

Eq SAA StableLab #1	
Serum Amyloid A:	2930 ug/ml
Reference Range:	0 - 7.5 ug/ml

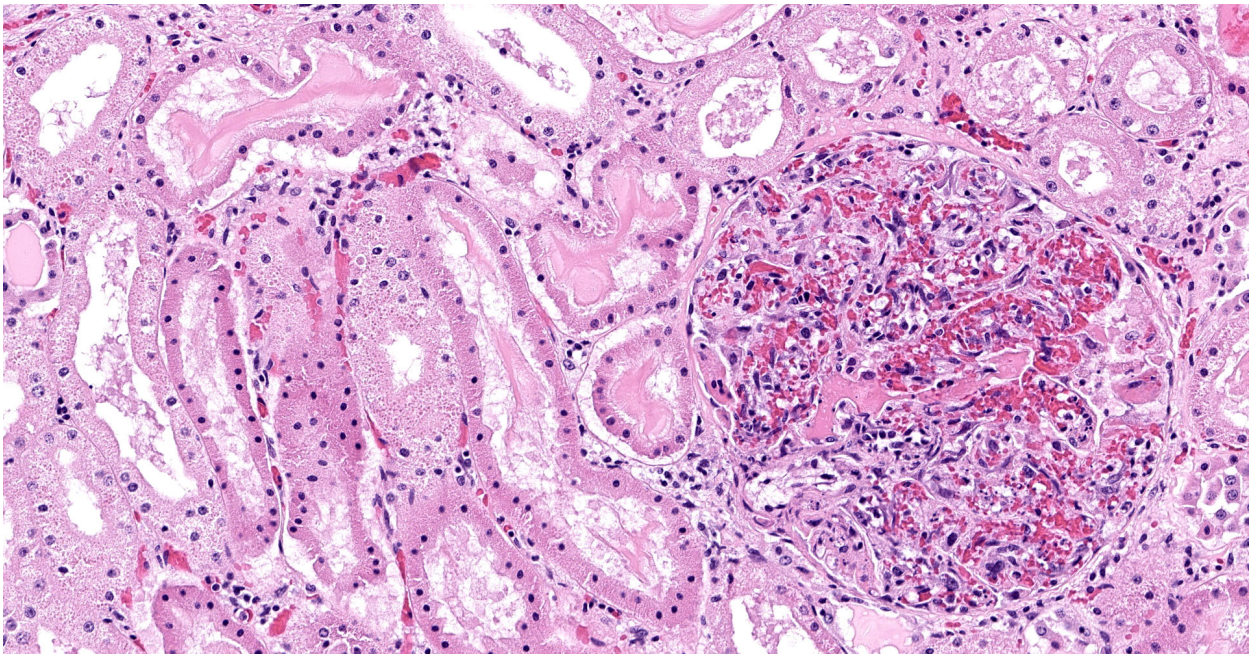
Fluid-wash flush #1	
Microscopic Eval:	A direct assessment of low cellularity and cytocentrifuge preparation are examined with a clear colorless background that contains abundant streaming mucus. Variably degenerate neutrophils predominate, many of which contain single and doublet cocci bacteria, which are also rarely observed extracellularly. Low numbers of leukophagic and moderately-markedly vacuolated macrophages are also observed, some of which contain amorphous refractile material intracellularly. Few pollen granules are noted extracellularly.
OVERALL	
Interpretation:	**Septic suppurative inflammation with small amount of refractile material**
Recommendations:	Bacterial culture and sensitivity
Comments:	The refractile material within the macrophages is of unknown origin or significance. This material may be silicosis or incidental sand aspiration from ground feeding.

Transmission Electron Microscopy
A sample of the renal cortex had been submitted for electron microscopy. Three glomeruli are evaluated. Throughout the glomerulus are large numbers of subepithelial, intramembranous, subendothelial, and perimesangial dense (immune) deposits. Mesangial cell interpositioning underneath the capillary loop basement membrane is observed in one location. Podocyte cell bodies are swollen and have foot process effacement. Rare lipid vacuoles are present in tubular epithelial cells.

Microbiology
Lung: A sample of the right cranial lung was submitted for aerobic bacterial culture and grew moderate numbers of *Streptococcus equi* subsp. *zooepidemicus* and very small numbers of *Staphylococcus aureus*.

Kidneys: Samples of kidney were submitted for aerobic bacterial culture and grew very small numbers of mixed growth including *Streptococcus equi* subsp. *zooepidemicus*, *Streptococcus viridans*, *Micrococcus* sp. and 2 colonies each two types of *Actinobacillus* sp.

Microscopic description:
One section of kidney is examined in which glomeruli are diffusely and globally enlarged and hypercellular with variable expansion and vacuolation of the mesangial matrix. Capillary lumina are frequently obstructed by swollen endothelial cells, thickened basement membranes and inflammatory cells predominantly composed of mononuclear cells and neutrophils. Segmental necrosis is prominent throughout glomeruli with



Kidney, horse. Glomeruli are hypercellular. There is expansion/effacement of capillary walls by variable combinations and concentrations of hemorrhage, fibrin, migrating neutrophils, cellular debris, and hypertrophic endothelial cells nuclei. Bowman's space is filled with high protein exudate and fibrin admixed with cellular debris. The epithelium lining adjacent tubules exhibits a variety of degenerative/necrotic changes including cytoplasmic granularity, pyknosis, and nuclear loss. (HE, 225X)

glomerular tufts and capillary walls being replaced by amorphous eosinophilic material (fibrin) admixed with nuclear fragments (karyolysis). Glomeruli tufts are occasionally shrunken, eosinophilic and hypocellular (obsolescence). Tubules are ectatic with the lumina often containing homogenous to finely granular eosinophilic material (protein), red blood cells, or sloughed epithelial cells. Tubular epithelium demonstrates one of the following changes: attenuation and flattening, hypereosinophilic epithelial cytoplasm with pyknotic nuclei (individual cell necrosis), microvacuolated cytoplasm, and slightly basophilic cytoplasm with vesiculate nuclei (regeneration). Multifocally tubules are replaced by fibrosis or separated by clear spaces (suggestive of edema) and hemorrhage. The perivascular interstitium is multifocally infiltrated by small aggregates of lymphocytes and plasma cells.

Special stains:

Kidney (H&E - 2-micron section): The glomeruli are hypercellular with scattered nuclear debris. Scant inflammatory cells (neutrophils) are noted within the affected glomeruli. There is also multifocal peri-glomerular fibrosis and multifocal interstitial lymphoplasmacytic inflammation. Occasionally, there are obsolescent glomeruli. Multifocally, renal tubules have attenuation and simplification of the renal tubular epithelium.

Kidney (Periodic acid-Schiff - 2-micron section): The glomeruli are hypercellular but the glomerular basement membrane appears unremarkable. Mesangial lysis is present in many glomeruli. Multifocally, the renal tubules are filled with protein droplets indicating protein leakage through the filtration barrier.

Kidney (Trichrome – 2-micron section): Glomerular fuschinophilic deposits are not observed.

Kidney (Jones Methenamine Silver Stain - 2-micron section): The presence of mesangial lysis is accentuated.

Kidney (PTAH): Intra-glomerular fibrin associated with hemorrhage is present within the glomerular tuft.

Immunohistochemistry:

Kidney (CD204): There were many CD204 positive macrophages within the affected glomeruli. This finding may be indicative of an immune response to mesangiolysis.

Kidney (Smooth Muscle Actin): Parietal cells within affected glomeruli had positive immunoreactivity for smooth muscle actin. This finding has been noted in different types of renal injury such as glomerulonephritis.

Contributor's morphologic diagnosis:

1. Kidney (light microscopy): Severe, diffuse, global, acute proliferate glomerulonephritis with mesangial lysis, hemorrhage, segmental glomerular necrosis, tubular epithelial attenuation, necrosis, regeneration and loss, and protein and cellular casts.

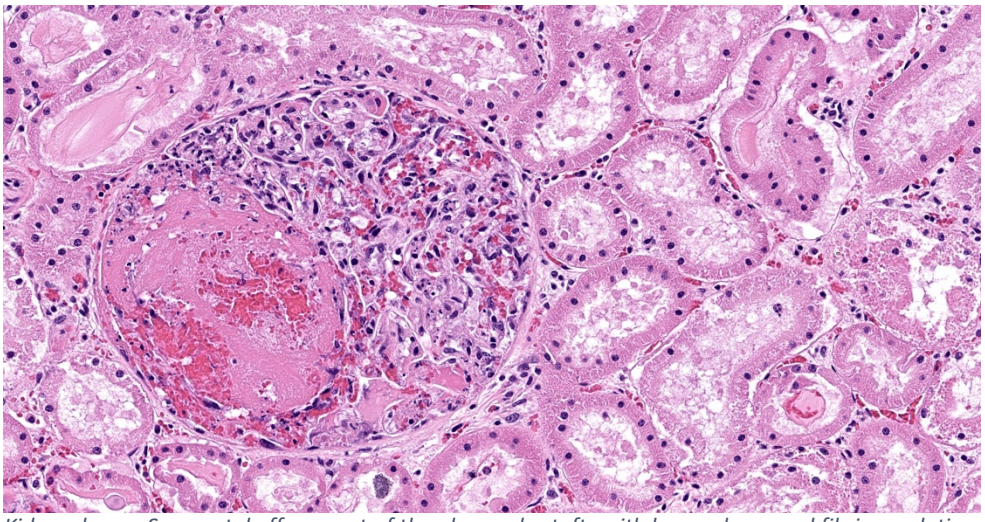
2. Kidney (TEM diagnosis): Severe, global, acute immune complex glomerulonephritis (ICGN) with subepithelial, intramembranous, subendothelial and peri-mesangial dense deposits.

3. Kidney: Mild, multifocal, chronic lymphoplasmacytic interstitial nephritis with fibrosis and focal, diffuse, glomerular obsolescence.

Contributor's comment:

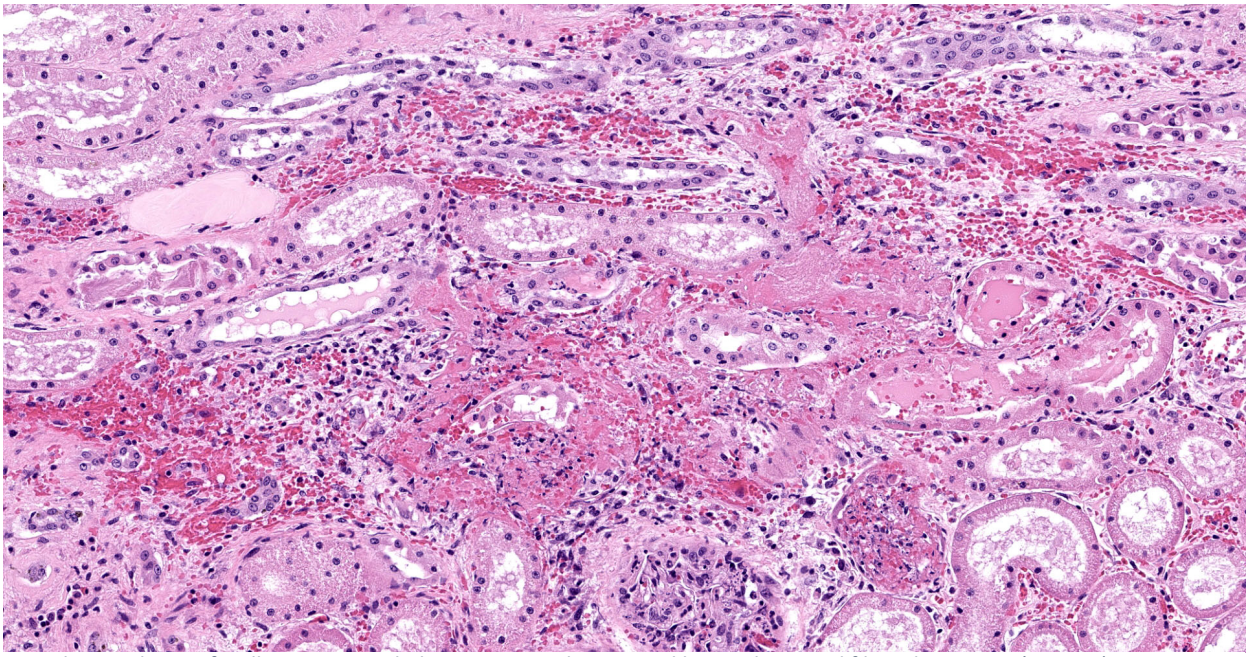
Etiologic agents and exact pathogenesis of immune-complex glomerulonephritis (ICGN) in animals are not well understood. It has been postulated that infectious diseases may provoke immune complex deposition within glomeruli resulting in glomerular pathology.[8] In horses, equine infectious anemia (EIA), *Streptococcus*- and herpesvirus antigen-antibody complexes have previously been documented to cause renal failure resulting from glomerulonephritis.[2,5] In humans, post-streptococcal glomerulonephritis (PSGN) is caused by prior infection with nephritogenic strains of beta-hemolytic streptococcus.

Human PSGN is associated with a previous skin, throat, or respiratory infection by group A streptococcus (*Streptococcus pyogenes*), albeit rare reports of groups C or G streptococcus are reported.[11] An epidemic outbreak in Nova Serrana, a small rural Brazilian community humans was associated with *Streptococcus zooepidemicus* transmitted during ingestions of unpasteurized, mastitic, cow milk.[1] *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is a β-hemolytic, Lancefield group C streptococcal bacterium.[3]



Kidney, horse. Segmental effacement of the glomerular tufts with hemorrhage and fibrin exudation. (HE, 257X)

S. zooepidemicus is known to be a commensal, opportunistic pathogen in horses associated with inflammatory airway disease in Thoroughbred racehorses[17,18], uterine infections in mares[9,13] and ulcerative keratitis[4]. Renal lesions develop as a consequence of either deposition of circulating antigen-

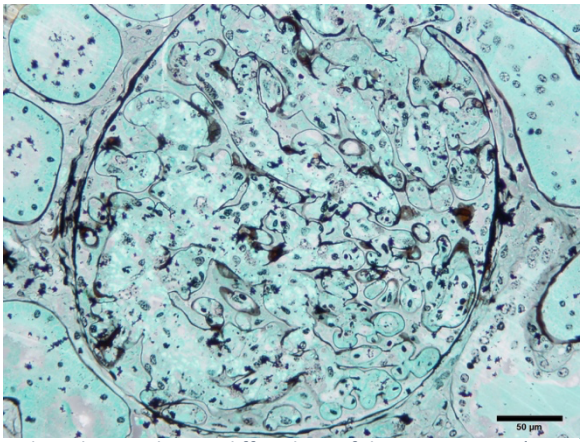


Liver, horse. There is focally extensive tubular necrosis and interstitial hemorrhage and fibrin deposition. (HE, 84X)

antibody complexes or from *in situ* reactions to streptococcal antigens. Historically implicated *S. zooepidemicus* antigens include M protein, R antigen, and a common protein antigen (CSCA) of *S.* equi and *S.* zooepidemicus.[6,14] Recent studies on gene expression of nephritis associated plasmin receptor (NAPlr, a glyceraldehyde 3-phosphate dehydrogenase), the streptococcal pyrogenic exotoxin (erythrotoxin) B (SPEB), and the SPEB zymogen precursor (zSPEG) in several streptococcal strains highlight the growing importance of these nephritogenic antigens.[11]

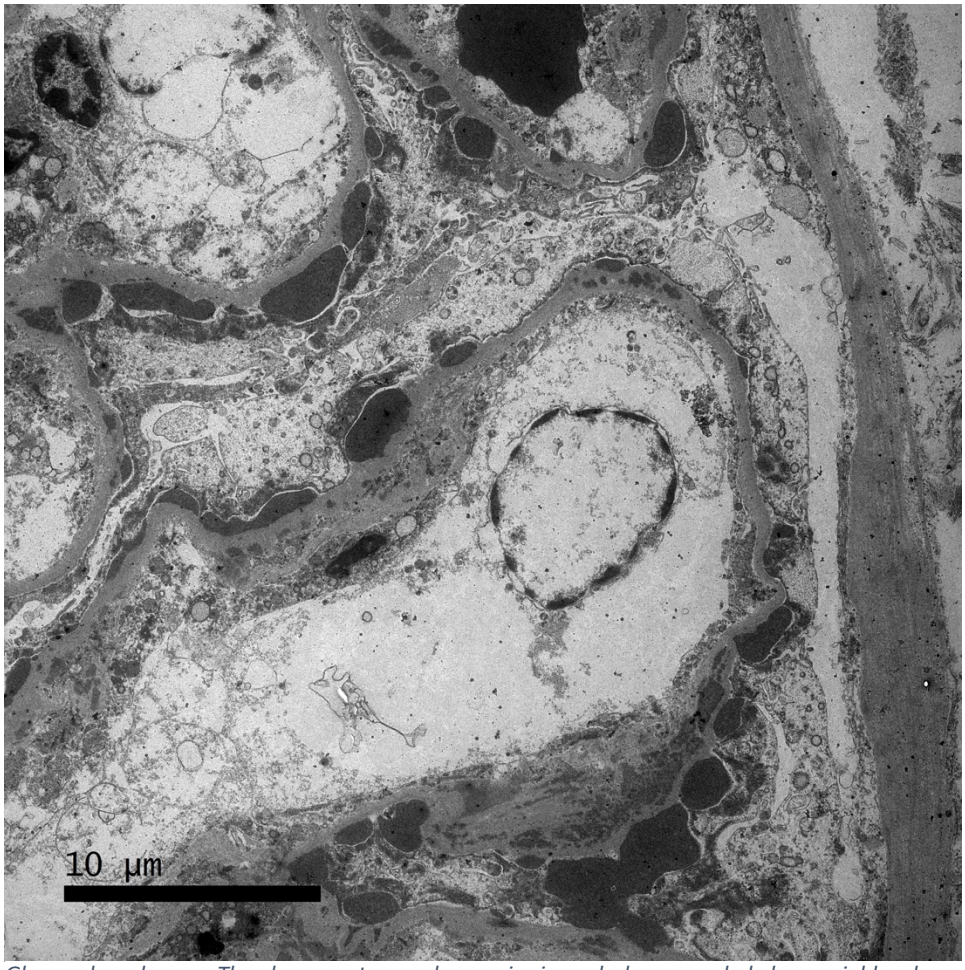
Light microscopic findings in post-streptococcal glomerulonephritis typically shows diffuse proliferative and exudative glomerulonephritis (GN). Glomeruli are enlarged with global endocapillary hypercellularity and variable numbers of neutrophils. With time, the endocapillary hypercellularity may transitions into a predominantly mesangial hypercellularity. Rarely do cases demonstrate membranoproliferative GN (MPGN). In humans, 60% of cases had histologic evidence of acute tubular injury.[10] Immunofluorescence microscopy has been extensively studied in people demonstrating that in the first 2-3 weeks, C3 and IgG deposits in the capillary walls and mesangial areas in a finely granular, "starry sky" pattern. With time, there is a predominance of C3 deposition in the mesangium, including subepithelial deposits within the mesangial "waist". Coarse to confluent granular staining along the glomerular capillary walls ("garland" pattern) creates the subepithelial "humps" that are the characteristic ultrastructural feature of this disease. These can be seen early or late in the disease.[11] Immunofluorescence was not pursued in this case due to the lack of available equine antibodies.

Other etiologic agents were considered in this case due to the light microscopic findings. A



Kidney, horse. There is diffuse lysis of the mesangium. (Jones Methenamine Silver, 400X)

comprehensive equine upper respiratory panel and an equine infectious anemia ELISA were performed to rule out herpesvirus and EIA. A toxic glomerular vasculopathy from envenomation was also deliberated due to the dilated and ruptured capillaries within glomeruli (mesangiolysis and capillary ballooning) with hyaline casts, in addition to the echinocytosis. Echinocytes (crenated erythrocytes) may be observed after envenomation, presumably secondary to the action of venom phospholipases; however, in horses, echinocytosis has been demonstrated with systemic electrolyte depletion (endurance exercise, furosemide treatment, diarrhea, and systemic disease).[7] Ultimately, dense deposits discounted this differential since dense deposits should not be present in a typical case of envenomation. Demonstration of IgG and C3 deposition in the renal glomerulus could provide confirmatory evidence that the glomerular pathology is due to an immune response. Regardless, pulmonary and renal culture results are highly suggestive of a post-streptococcal glomerulonephritis.



Glomerulus, horse. The basement membrane is irregularly expanded by variably dense intramembranous and subepithelial dense deposits. There is effacement of secondary podocyte foot processes and vacuolation and expansion of endothelial cell cytoplasm of endothelial cells at upper left). (TEM, bar – 10um)

Contributing Institution:
University of California, Davis
https://www.vetmed.ucdavis.edu/hospital/support-services/lab-services/anatomic-pathology-service

JPC diagnosis:
Kidney: Glomerulonephritis, proliferative and with superimposed microangiopathy, diffuse, severe, with tubular degeneration, necrosis, and regeneration, proteinosis, and red cell casts.

JPC comment:
The contributor provides a concise summary of immune-complex glomerulonephritis. In addition to post-streptococcal glomerulonephritis, the most common causes of type III hypersensitivity reactions include serum sickness, systemic lupus erythematosus, farmer's lung (hypersensitivity pneumonitis), and rheumatoid arthritis. Common to all of these etiologies is a similar three step process that leads to tissue damage. First, endogenous or exogenous antigen triggers

antibody formation, usually within 4-10 days. Antibodies bind antigen and form circulating complexes. Second, when there is a slight excess of antigen, the formed immune complexes are smaller and filter out of circulation more easily, typically in tissues where a filtrate is formed from blood, like synovial fluid or urine. This explains how joints and glomeruli are primary targets of immune complex deposition. Thirdly, the classical pathway to complement activation leads to the release of C3a and C5a (anaphylatoxins) that recruit neutrophils and macrophages which cause inflammatory damage to tissues.[16]

Specific to the proposed pathogenesis of this case, Streptococcus equi subsp zooepidemicus has a number of identified fibrinonectin binding proteins (FNZ/FNE, FNZ2/FNEB, SFS), M-like proteins (SzM/SeM, SzPSe/SzP), immunoglobulin binding proteins (ZAG), toxins (streptolysin S), and a number of superantigens (SpeK, SpeL, SzeN, SzeP, SzeF). The combination of superantigens and immunoglobulin binding proteins make this bacterium a potent source of circulating immune complexes.[15]

Human medicine has a long history of documenting acute post-streptococcal glomerulonephritis (APSGN), but usually caused by a group A β-haemolytic *Streptococcal* infection. Starting in the mid-1900's, the incidence of APSGN cases began to decline, attributed to improved standards of living and socioeconomic conditions in most developed countries. However, a recent surge of methicillin-resistant *Staphyloccocus aureus* infections over the last 20 years has changed the epidemiology of post-infection glomerulonephritides. While the number of veterinary cases of immune complex glomerulonephritis attributed to *Staphylococcus aureus* is unknown in large part, the shift observed in human clinical medicine may portend a shift in our patients as well.[12]

A portion of the conference discussion centered on the capillary thrombi seen in glomeruli. Evaluated together with the TEM images revealing extensive endothelial damage, this consensus was that this horse had a superimposed thrombotic microangiopathy (TMA), affecting the glomerular and peritubular capillaries.

Leptospirosis was discussed as a possible differential diagnosis in this case. The moderator more often sees tubulocentric damage associated with leptospira, but pathologists located in different regions of the United States have seen glomerular-centric cases of equine leptospirosis, which also lead to glomerular microthrombosis.

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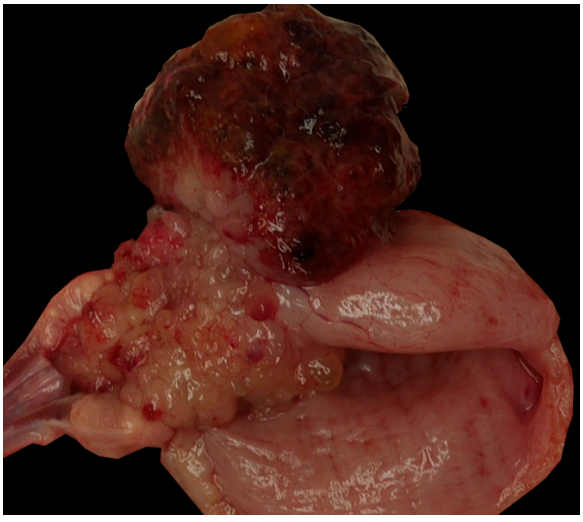
CASE 4: 8002353 4001 33 ABL (4136107-00)

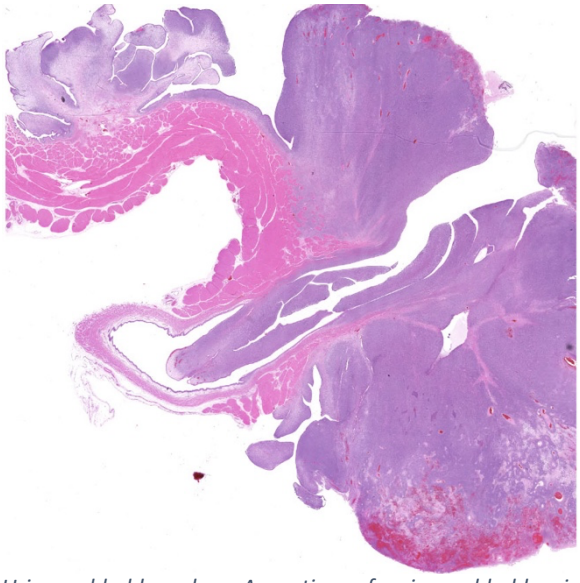
Signalment:
14-month-old male Beagle dog (*Canis familiaris)*

History:
The dog was part of a 13-week toxicity study. On Day 68 of the study, the animal appeared to be in some discomfort, voided a small amount of red urine in a discontinuous stream, and then exhibited transient tremors and adopted the "prayer stance" suggestive of abdominal pain. The animal was subsequently removed from study and humanely euthanized.

Gross Pathology:
Expanding and partially effacing the urinary bladder mucosa in the area of the trigone and the prostate was a firm 5.5x4x3 cm, variably pale tan to dark red, multilobular mass that protruded and partially obstructed the neck of the bladder and urethral lumen. The bladder lumen was dilated, and the mucosa of the adjacent bladder and urethra were discolored dark red and were mildly gelatinous (edematous). The left kidney was mildly enlarged, and the left renal pelvis and ureter were moderately dilated (hydronephrosis and hydroureter).



Urinary bladder, dog. There is a large polypoid neoplasm effacing the wall and extending into the lumen within the trigone. (Photo courtesy of: Takeda Pharmaceuticals International Co., 35 Landsdowne St. Cambridge, MA 02139 https://www.takeda.com/)

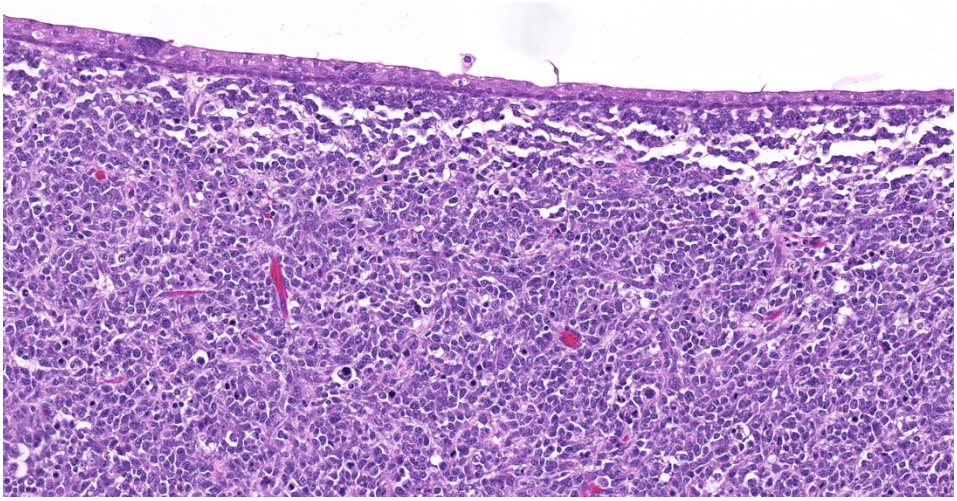


Urinary bladder, dog. A section of urinary bladder is submitted for examination. The submucosa is diffusely expanded by an infiltrative densely cellular neoplasm which throws the overlying mucosa into variably sized polyps and focally effaces the underlying smooth muscle. (HE, 6X)

Laboratory results:
Prior to study initiation, urinalysis, hematology, and serum chemistry was performed. Notable results on urinalysis consisted of 3+ to 4+ blood and protein, with abundant red blood cells and fewer white blood cells present in urine sediment. On hematology and serum chemistry were minimally decreased red blood cell parameters (red blood cell count, hemoglobin) and evidence of inflammation consisting of mildly elevated total white blood cells attributable to increases in neutrophils and monocytes, and decreased albumin to globulin ratio attributable to both increased serum globulins and decreased serum albumin. These analyses were repeated on Day 68 of the study and the results were similar to the pre-study timepoint.

Microscopic description:
Urinary bladder: Partially effacing the wall of the bladder in the area of the trigone, elevating the overlying urothelium, and extending into the bladder and partially obstructing the urethral lumen, was a poorly demarcated, unencapsulated neoplasm composed of cells exhibiting various morphologies. The majority were small, polygonal, undifferentiated cells with minimal cytoplasm, compact round nuclei with moderate mitotic rate (7/10 HPF), in nests and packets separated by fine fibrovascular stroma. In some areas, particularly in areas underlying the urothelial mucosa, the neoplasm was composed of sheets of sparse pleiomorphic spindle to stellate cells with low mitotic rate (<1/10/HPF) embedded in abundant myxomatous matrix. Elongated, rarely multinucleated cells with large round to oval vesicular nuclei and visible cytoplasmic striations ("strap cells") were occasionally present in these areas. Minimal mixed inflammatory cell infiltrates were scattered throughout the section. The superficial aspect of the tumor that was protruding into the bladder lumen was necrotic and hemorrhagic, and the overlying urothelium was absent (ulcerated). In other areas the urothelium was variably hyperplastic or vacuolated.

Contributor's morphologic diagnosis:
Urinary bladder: Botryoid rhabdomyosarcoma



Urinary bladder, dog. Neoplastic cells are densely packed in small streams and bundles with small amounts of fibrillar cytoplasm, prominent nuclei and nucleoli, and a high mitotic rate. (HE 400X)

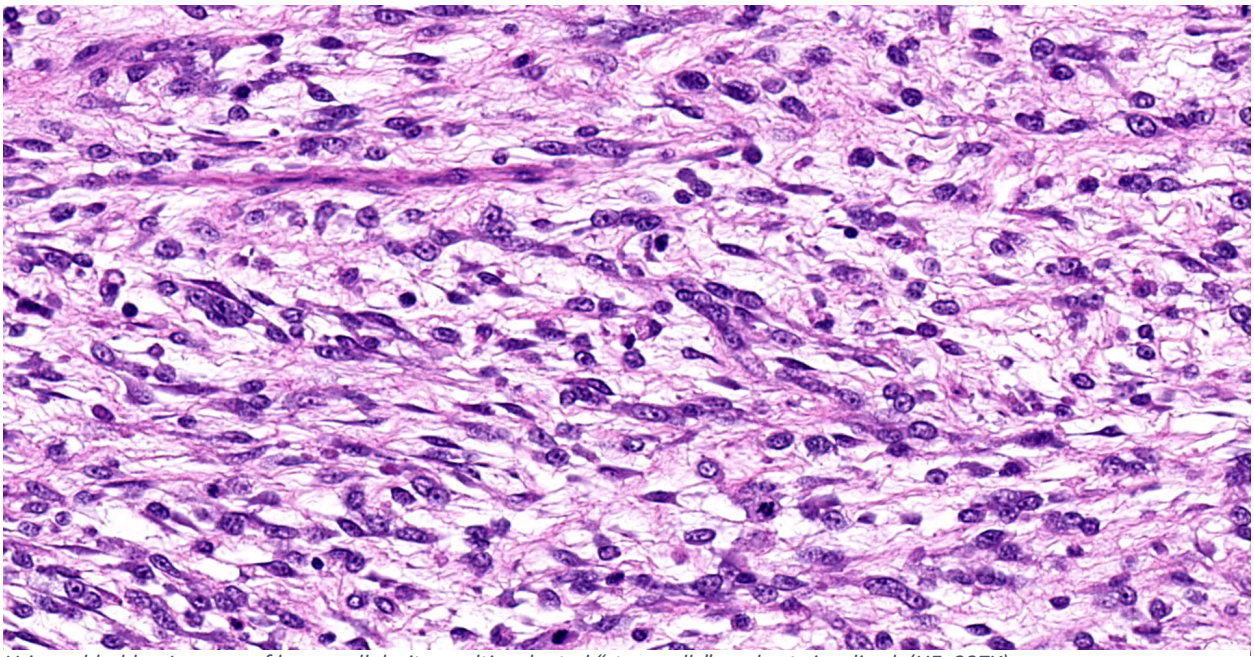
Contributor's comment:
This is the classic presentation for spontaneous canine botryoid rhabdomyosarcoma: a multilobular "grape-like" mass at the trigone of the bladder in a young dog. These tumors are rare overall (<0.5% of all bladder tumors) but are most commonly reported in dogs less than two years old with some predilection for larger breeds such as Saint Bernards. However, sporadic reports of occurrence in small breeds and in other species (horse) are also present in the literature.[15] Other types of rhabdomyosarcoma include embryonal, alveolar, or pleiomorphic. The clinical significance of the different histologic types in the dog is unknown; in humans the alveolar variant is associated with poor prognosis.[2]

Although to the authors knowledge botryoid rhabdomyosarcoma has not been reported in the beagle, there is one case report of a beagle with cardiac alveolar rhadbdomyoma.

Histologically, the cells comprising botryoid rhabdomyosarcoma resemble variably differentiated myoblasts (small polygonal cells) to skeletal myocytes, and the presence of well-differentiated strap cells containing cross-striations is characteristic. Consistent with a proposed origin of remnant embryonic myoblastic cells, these tumors may be variably positive for immunohistochemistry markers of mesenchymal cells and skeletal muscle based on the degree of differentiation (vimentin, desmin, S100, myoglobin, myogenin, or MyoD immunohistochemistry and PTAH special stain), and are negative for markers of epithelial cells or smooth muscle (cytokeratin, αSMA).[8] This case was strongly desmin positive, multifocally PTAH positive only in more differentiated areas of the tumor, inconclusive for myoglobin, and αSMA negative.

Metastasis has been reported with botryoid rhabdomyosarcomas but is generally considered rare with this tumor type. However, given the location of the tumor surgical resection is often challenging and local complications such as urinary obstruction are common clinical sequela.[1,13] Other common clinical manifestations, some of which were present in this dog, are cystitis and hydronephrosis/hydroureter, as well as peripheral fibromatosis and hypertrophic osteopathy.[16]

The macroscopic, multinodular appearance of a mass in the trigone of the bladder of a young dog is highly suggestive of botryoid



Urinary bladder. In areas of lower cellularity, multinucleated "strap cells" are best visualized. (HE, 397X)

rhabdomyosarcoma. Differential diagnoses for a mass in this location may include transitional cell carcinoma, leiomyosarcoma, other primary or secondary tumors reported in the bladder of dogs (e.g. histiocytic sarcoma, lymphoma, osteosarcoma, hemangiosarcoma, etc.), inflammatory cystitis, or extension of prostatic neoplasia, e.g. squamous cell carcinoma.[6,17] Unless the rhabdomyosarcoma is highly undifferentiated, each of these has histologically distinct features.

Contributing Institution:
Takeda Pharmaceuticals International Co.
35 Landsdowne St. Cambridge, MA 02139
https://www.takeda.com/

JPC diagnosis:
Urinary bladder: Botryoid rhabdomyosarcoma.

JPC comment:
Canine rhabdomyosarcoma is most often found in laryngeal and cardiac tissues, but the botryoid variant arises most commonly in the trigone of the urinary bladder and urethra. However, it is also rarely reported to arise in the uterus and vagina.

In human medicine, an additional differential diagnosis is fibroepithelial polyp. A recent case illustrated a growth in the urinary bladder with prominent botryoid features and an embryonal rhabdomyosarcoma pattern, though lacking nuclear or cellular atypia. Sequencing the affected tissue revealed two pathogenic mutations in the dicer1 ribonuclease III (*DICER1*) gene. While we rarely sequence tissues for routine diagnosis, it remains possible that this mutation or similar may cause pathology that mimics botryoid rhabdomyosarcoma.[5]

Rhabdomyosarcoma arises in many species, in a variety of tissues. In horses, botryoid rhabdomyosarcoma has been reported in the uterus and urinary bladder, with embryonal rhabdomyosarcomas arising most often in the tongue and limb muscles.[3] Rhabdomyosarcoma has also been reported in cats[10], sheep[4], goats[9], mice (rare natural occurrences; frequent in experimental models)[12], pigs[4], cattle[4], and brook trout.[14]

As correctly identified by the resident, the moderator emphasized that the correct terminology is now "urothelial cell", which replaces "transitional cell" when referencing the urinary epithelial cell layer.

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