Joint Pathology Center
Veterinary Pathology Services

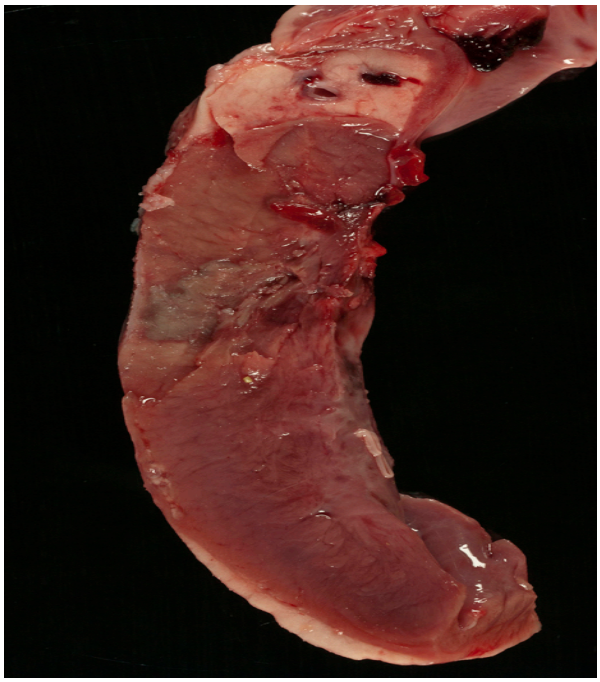
WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 17

26 February 2014

CASE I: 48772-A (JPC 4033980).

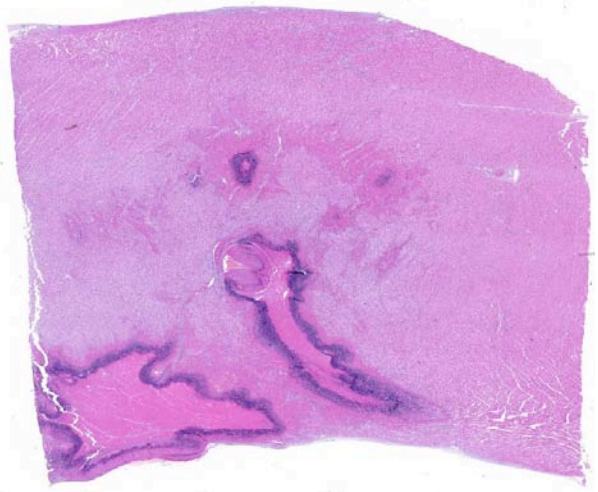
Signalment: 1-year-old intact male Romney sheep (*Ovis aries*).



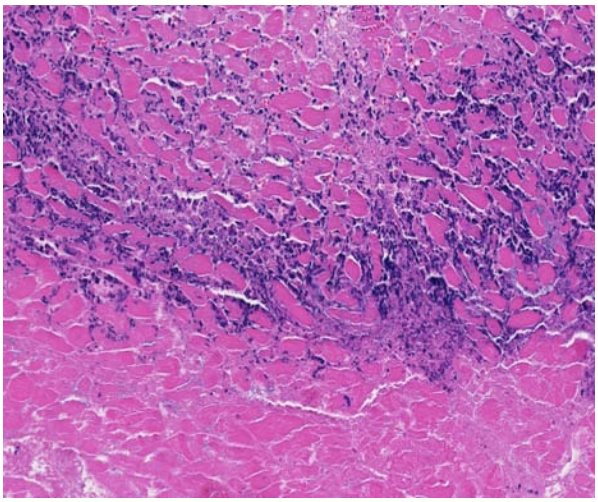
1-1. Heart, sheep: The incised myocardium contained several sharply demarcated, irregular-shaped 2-3 cm diameter foci which varied from pale tan to grey/green, and often had a red margin. (Photo courtesy of: IVABS, Massey University, Palmerston North, New Zealand http://www.massey.ac.nz)

History: This sheep was one of several rams purchased 10 days earlier from the sale yards for a research trial. The animals had been transported for approximately 2 hours by truck to the sale yards and had been drenched with an anthelmintic on two occasions during the 10 days since purchase. This animal was found dead in its paddock without showing any previous signs of illness. The vaccination history was unknown.

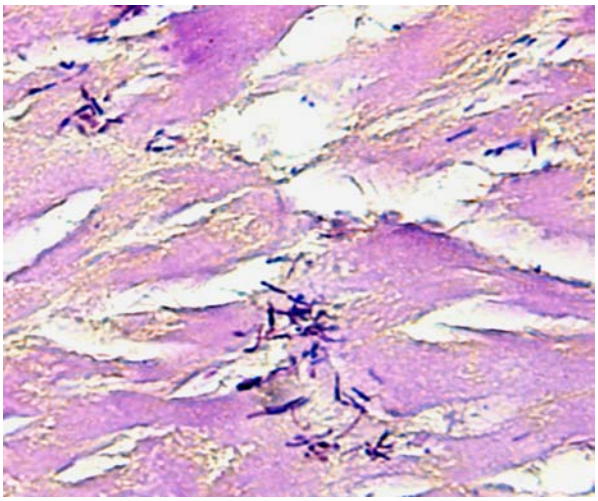
Gross Pathology: The ram was in good physical condition but was relatively small for its age, weighing 36.5kg. Although the abdomen was distended and blood-stained froth was exuding from the nostrils, there was little evidence of post-mortem decomposition at the time of examination. The lungs were diffusely red and edematous and contained multiple 2-3 mm dark red foci. The thoracic cavity contained approximately 1.5 liters of red fluid. Clear, yellow fluid was also present in the pericardial sac and the epicardial surface of the heart was almost completely covered by a thick, loosely adherent sheet of fibrin. On cut surface, the myocardium contained several sharply demarcated, irregular 2-3 cm diameter foci which varied from pale tan to grey/green, and often had a red margin. One focus involved the interventricular septum and extended to the



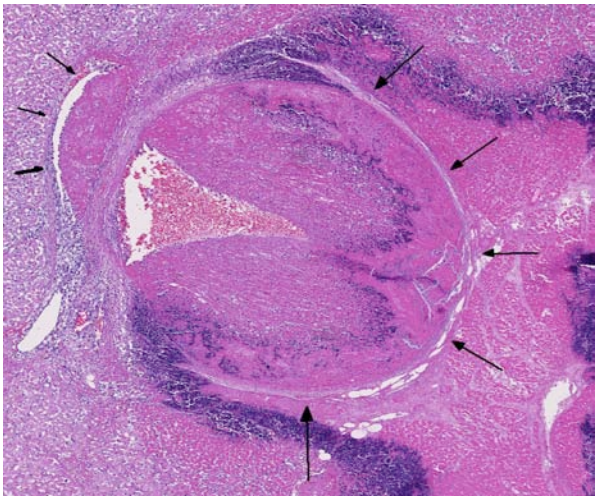
1-2. Heart, sheep: The myocardium contains several extensive areas of coagulative necrosis outlined by a dark blue band of cellular debris. (HE 0.63X)



1-3. Heart, sheep: At higher magnification, areas of coagulative necrosis (lower left) are surrounded by a band of degenerate neutrophils and abundant cellular debris which surrounds and separates adjacent degenerating myofibers. (HE 200X)



1-4. Heart, sheep: Moderate numbers of robust bacilli are scattered throughout the areas of coagulative necrosis. (Brown and Brenn 400X)



1-5. Heart, sheep: One area of necrosis incorporates a partially thrombosed arteriole (large arrows), and a smaller thrombosed vein (smaller arrows). (HE 160X)

luminal surface of the right ventricle, where it was covered by a fibrin thrombus.

Histopathologic Description: The focal lesions observed grossly in the heart consisted of acute coagulation necrosis surrounded by a thick layer of degenerate neutrophils. Within the necrotic areas, the interstitium was expanded with fibrinous exudate and many interstitial blood vessels and lymphatics contained fibrin thrombi. Large numbers of long, rod-shaped, gram-positive bacteria were present in necrotic areas, particularly near the margins. In surrounding areas of myocardium there was variable acute necrosis of myocardial fibers, neutrophilic interstitial infiltration and edema. The epicardium in some areas was markedly thickened with a layer of fibrin and moderate to large numbers of neutrophils. Necrotic foci that extended to the endocardial surface were covered with a layer of fibrin containing degenerate neutrophils.

Immunohistochemistry for *C. chauvoei*, *C. septicum*, *C. novyi* and *C. sordelli* performed at CAHFSL, San Bernardino (courtesy of Dr. F. Uzal) revealed organisms reacting positively for both *C. chauvoei* and *C. septicum*.

In the lungs, there was diffuse congestion of alveolar capillaries and proteinaceous alveolar

Table 1: Select clostridial diseases in veterinary species.[1,4,5]

Name	Species
C. perfringens type:	
A (α toxin)	• Enterocolitis in many species: ○ Foals, piglets, lambs, calves ○ Necrotic enteritis- chickens ○ Hemorrhagic canine gastroenteritis ○ Hemorrhagic bowel syndrome- dairy cattle • Yellow lamb disease- IV hemolysis (rare) • Gas gangrene- humans and animals
B (α, β, ε toxins)	• Enterocolitis in Europe, South Africa, Middle East ○ Lamb dysentery; similar condition in neonatal calves
C (α, β toxins)	• Enterocolitis in many species: ○ "Struck" in adult sheep in the UK; similar condition in goats and feedlot cattle ○ Necrohemorrhagic enteritis- lambs, calves, piglets, foals
D (α, ε toxins)	• Enterocolitis: ○ overeating disease/pulpy kidney- sheep, goats, cattle • Focal symmetric encephalomalacia- sheep and (rarely) goats
E (α, ι toxins)	• Enterotoxemia- calves, lambs, guinea pigs, rabbits
C. haemolyticum	• Bovine bacillary hemoglobinuria ("redwater") 2º to fluke migration
C. septicum	• Necrohemorrhagic abomasitis ("braxy") • Malignant edema • Postparturient vulvovagnitis and metritis in dairy cattle
C. novyi	• Hepatic necrosis ("black disease") 2º to fluke migration • Wound infections
C. chauvoei	• Necrotizing myositis ("blackleg") in cattle and sheep
C. colinum	• Ulcerative enteritis- "quail disease" in gallinaceous birds
C. spiroforme	• Spontaneous or antibiotic-induced enterotoxemia in rabbits and rodents
C. difficile	• Spontaneous or antibiotic-induced enterocolitis in hamsters, rabbits, guinea pigs, dogs, swine and horses
C. piliforme	• Necrotizing enteritis/colitis, hepatitis, myocarditis in many species (Tyzzer's disease) • Obligate intracellular, gram-negative
C. carnis, C. histolyticum	• Wound infections in many species
C. tetani	• Tetanus- muscle spasms, stiff gait, rigid posture, trismus (lockjaw)
C. botulinum	• Botulism- flaccid paralysis→ respiratory paralysis

oedema. There was also multifocal intra-alveolar haemorrhage and fibrinous exudation. Moderate numbers of neutrophils were present in the interstitium and in many alveoli.

Contributor's Morphologic Diagnosis: 1. Acute multifocal necrotising myocarditis.
2. Acute diffuse fibrinous epicarditis

Contributor's Comment: Clostridial myocarditis is a rare disease of sheep. To our knowledge, it has only been reported once previously (in Australia)[2] and is probably analogous to the myocardial form of "blackleg" in cattle, which is caused by *Clostridium chauvoei.*[3] No lesions were present in skeletal muscles of the ram, supporting primary myocardial involvement. The myocardial lesions were characterized by a greater neutrophilic response than would be expected in clostridial myositis involving skeletal muscles. This was also a feature of lambs and calves in previous reports of clostridial myocarditis.[2,3] An unexpected finding in this case was the presence of both *C. chauvoei* and *C. septicum* in the myocardial lesions, suggesting that latent spores of both organisms were present in the myocardium.

In published reports of clostridial myocarditis in lambs and calves, potential risk factors included lush pasture growth following a period of high rainfall, and stress associated with yarding and management procedures. Both factors existed in the present case. It is possible that the stress involved in recent transport and yarding for drenching caused focal catecholamine-induced myocardial necrosis, allowing germination of latent clostridial spores. Vaccination with a 5 in 1 clostridial vaccine would have been expected to prevent the disease, suggesting that this ram had not been vaccinated.

JPC Diagnosis: Heart: Myocarditis, necrotizing, multifocal to coalescing, moderate with large colonies of bacilli, necrotizing arteritis and thrombosis.

Conference Comment: There is moderate slide variation in this case; some sections contain a large arteriole adjacent to a focus of myocardial necrosis which is 80-90% occluded by an organizing fibrin thrombus, while in other sections, necrotizing arteritis and fibrin thrombi

are limited to smaller coronary vessels within the endocardium. The presence of these fibrin thrombi supports the interpretation of the well demarcated areas of necrosis as infarcts. Additionally, most sections of myocardium contain rare protozoal cysts packed with numerous bradyzoites (*Sacrocystis* sp.), without evidence of associated inflammation; infection with *Sarcocystis* sp. is a common, incidental finding in the skeletal and cardiac muscle of ruminants.[4] Histochemical staining with a Giemsa as well as a Gram stain highlighted the presence of numerous gram-positive bacilli with multifocal subterminal spores, a characteristic feature of *Clostridium* sp.

Members of the genus *Clostridium* are spore forming, gram-positive, anaerobic bacilli, whose spores are ubiquitous in soil, highly resistant to environmental changes and disinfectants, and able to persist in the environment for many years. Germination of spores, which are often ingested by ruminants during grazing, requires specific conditions, including low oxygen levels and an alkaline pH; necrotic lesions and penetrating wounds provide appropriate conditions for growth of clostridial spores. Clostridia typically produce disease via production of exotoxins which cause widespread necrosis with edema and extensive gas formation.[1,4,5] See table 1 for an abbreviated list of *Clostridium* sp. important in veterinary medicine.

Contributing Institution: IVABS
Massey University
Palmerston North, New Zealand
http://www.massey.ac.nz

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CASE II: AFIP-WSC H8674 39 (JPC 3165179).

Signalment: 18-year-old female Indian origin rhesus macaque, (*Macaca mulatta*).

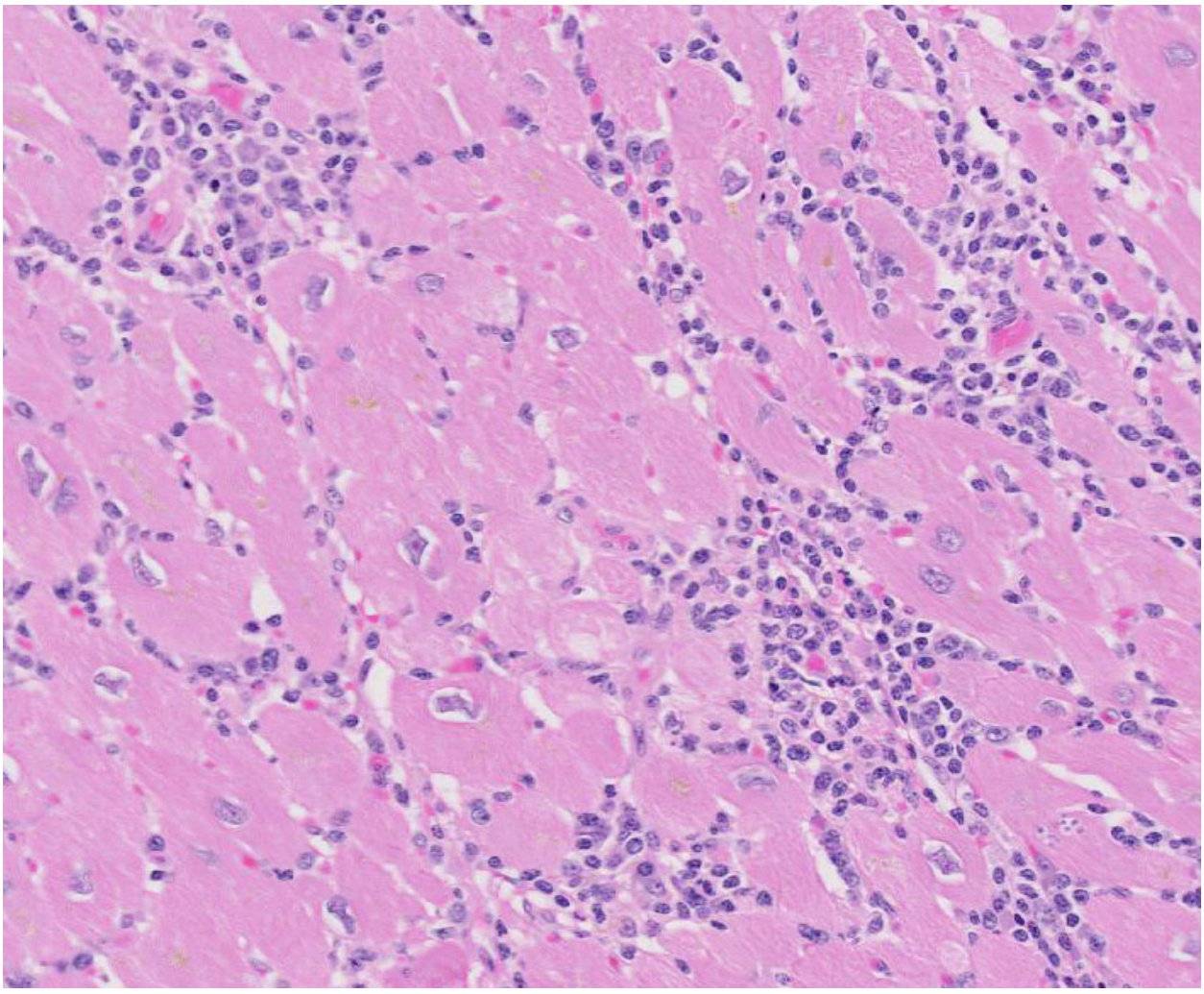
History: Female with 7-week-old infant sedated (ketamine) for routine blood work developed seizures, high heart rate, pale mucous membranes, fixed pupils, euthanized.

Gross Pathology: Minimal pitting edema in the subcutaneous tissues; ~ 300 ml straw colored fluid within the abdominal cavity; ~ 350 ml straw colored fluid within the thoracic cavity with collapsed lung lobes and the diaphragm bulging into the abdominal cavity.

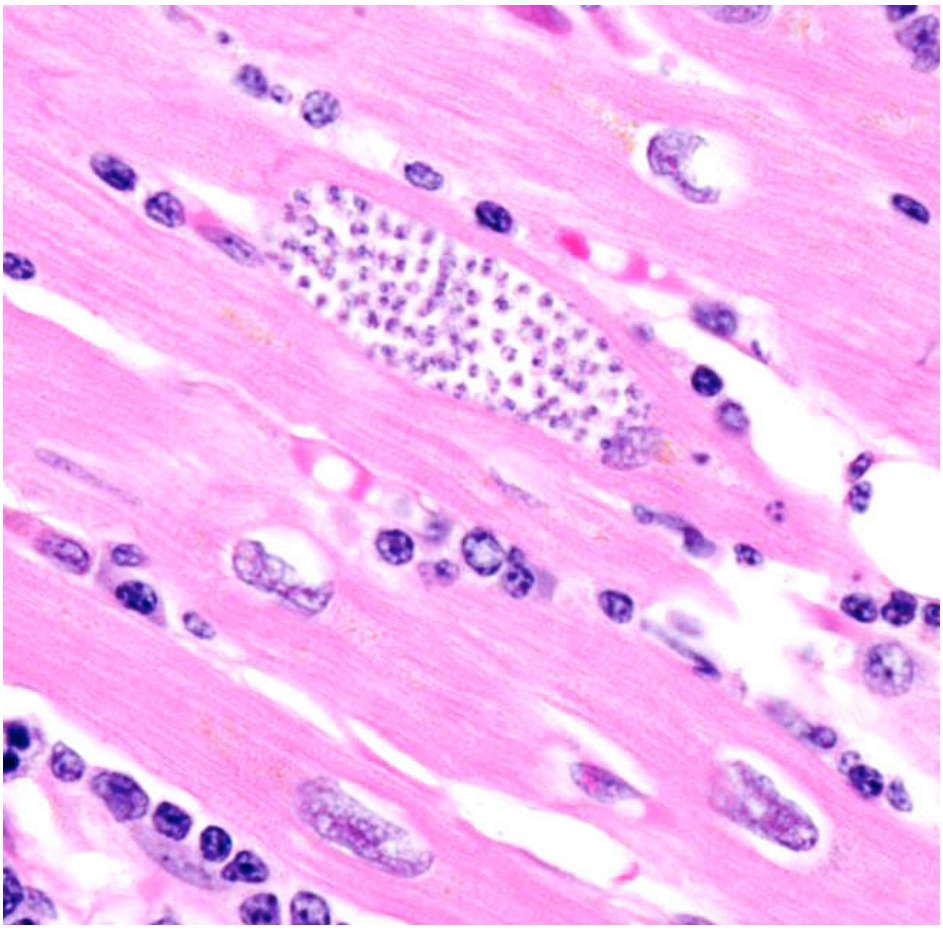
Histopathologic Description: A multifocal to diffuse inflammatory process is evident within the myocardium. There are large numbers of lymphocytes, plasma cells, macrophages and smaller areas of neutrophil infiltration. Many cardiomyocytes contain protozoal organisms consistent with amastigotes of *Trypanosoma cruzi*. The cardiomyocytes containing amastigotes have lost sarcomeres and are undergoing degeneration.

Contributor's Morphologic Diagnosis: Heart, diffuse, marked, chronic active inflammation, myocardium with intracellular protozoal organisms consistent with *Trypanosoma cruzi*.

Contributor's Comment: *Trypanosoma cruzi,* the etiologic agent of Chagas disease, is endemic within portions of the southern United States, including Texas and the colony where this case occurred.[4-7] Nonhuman primates housed outdoors within areas endemic for this parasite should be monitored with increased vigilance for contact



2-1. Heart, rhesus monkey: Diffusely, numerous lymphocytes, plasma cells, and fewer histiocytes surround and separate cardiomyocytes. (HE 180X)



2-2. Heart, rhesus monkey: Occasional cardiomyocytes contain numerous 2-3 µ m amastigotes within their cytoplasm. The surrounding cardiomyocytes contain small amounts of perinuclear lipofuscin. (HE 400X)

with both vectors (triatomine beetles such as the kissing bug, assassin bug, reduviid beetle)[4-6] and reservoir hosts (sylvatic small mammals such as rodents and opossums).[6] It has been postulated that the alert nature of rhesus macaques makes feeding by triatomine beetles unlikely.[6]

A review of the database of pathologic lesions diagnosed within our colony over the last thirty years revealed no other cases of severe trypanosomiasis, and even mild cases were rare. Pest control procedures are well established and vigilantly enforced; no increased incidence of pests (either rodents or triatomine beetles) was noted before or since the identification of this case. In May of 2005 a survey of sixty apparently normal animals from this colony revealed a single animal with a positive culture. Serendipitously, the animal presented in this case was included in that study and had a negative culture at that time. This case is considered unusual, due to the high number of leishmanial forms of *Trypanosoma cruzi* organisms that are present within pseudocysts in individual cardiomyocytes as well as the abundant inflammatory reaction. At locations where pseudocysts have ruptured a suppurative inflammatory reaction is observed; however, the primary inflammatory population within this lesion is mononuclear, composed primarily of lymphocytes, macrophages and plasma cells. The affected animal was in late pregnancy when she presented for annual physical examination. During routine sedation, the animal became cyanotic and when emergency drugs failed to elicit any positive response the animal was euthanized. Potential immune suppression associated with pregnancy or mildly advanced age may have resulted in the development of this protozoal infection; alternatively, a concentrated mucocutaneous exposure secondary to ingestion of the vector may have led to this disease.[5,6]

JPC Diagnosis: Heart: Myocarditis, necrotizing, lymphoplasmacytic and histiocytic, subacute, diffuse, severe with numerous intramyocytic amastigotes.

Conference Comment: Trypanosomes are hemoflagellate protozoans that typically cause cardiac disease; *Trypanosoma cruzi* is the major etiologic agent of American trypanosomiasis, while *T. brucei* and *T. congolense* (among others) cause African trypanosomiasis. Humans, as well as numerous domestic and wild mammalian species within the Western Hemisphere, are susceptible to *T. cruzi*, although disease is most

common in South and Central America. As noted by the contributor, transmission is typically associated with intermediate arthropod vectors, particularly *Triatoma* sp. (i.e., Reduviidae, or the "kissing bug"); stercorarian transmission occurs when feces from an infected bug contaminates the bite wound or nearby abrasions.

T. cruzi occurs in three morphologic forms. Epimastigotes are found within the arthropod vector, while the extracellular trypomastigote (blood form) and intracellular amastigote (tissue form) occur within the mammalian host. The amastigote contains a large round nucleus and a rod-like kinetoplast similar to that of *Leishmania* sp.[7,8] Leishmanial and trypanosomal amastigotes are anecdotally described as having rod-shaped kinetoplasts oriented perpendicular and parallel to the long axis of the oval nucleus, respectively; however, most current references simply describe a juxtanuclear kinetoplast composed of DNA and representative of the mitochondrial genome,[3] which led conference participants to conclude that kinetoplast orientation relative to the nucleus is not an accurate or reliable method for distinguishing *Trypanosoma* sp. from *Leishmania* sp. Readers may refer to WSC 2011-2012, Conference 18, case 1 for further discussion of *Trypanosoma cruzi*.

African trypanosomiasis, or sleeping sickness, also occurs in humans and a variety of animal species. It is transmitted through the saliva of the tsetse fly (*Glossina* sp.). Unlike *T. cruzi*, African trypanosomes are capable of continuous antigenic variation of their outer glycoprotein coat in order to evade the host immune response. They also produce sialidases, which hydrolyze host cell membrane sialic acid and facilitate invasion. In addition to myocarditis, ocular involvement, anemia, thrombocytopenia and even disseminated intravascular coagulation have been reported in association with African trypanosomiasis.[8]

In this case, anesthesia likely led to decompensation of existing cardiac disease. There is multifocal cardiomyocyte hypertrophy, characterized by fiber thickening and change of the nuclei from spindle/cigar shaped to "box car" like (rectangular). Additionally, cardiomyocytes often contain aggregates of golden-brown granular pigment, interpreted as lipofuscin, which represents intralysosomal accumulation of cellular debris (i.e., residual bodies).[9] There is also mild slide variation, with varying degrees of myocardial fibrosis (demonstrated with a Masson's trichrome stain) depending on the section; however, fibrosis is a common background finding in macaque hearts[2] and may not be a direct result of the trypanosome infection. Conference participants briefly discussed the differential diagnosis for the gross and histological findings, including leishmaniasis, toxoplasmosis, sarcocystosis, encephalitozoonosis and African histoplasmosis. Histochemical staining with giemsa highlights the presence of moderate numbers of intracellular cardiomyocyte protozoal amastigotes with a rod-like kinetoplast, consistent with trypanosomiasis or leishmaniasis. It can be difficult to differentiate *Trypanosoma* spp. from *Leishmania* spp.; however, the kinetoplast of *T. cruzi* is typically larger, and leishmanial amastigotes tend to concentrate in the phagocytic cells of the skin, mouth, nose and throat, or in the macrophages of the reticuloendothelial system, which are all uncommon sites for *T. cruzi*.[1] Based on the species, its geographic location and the anatomic location of the lesions, trypanosomiasis is the most likely diagnosis; however, since additional diagnostic testing, such as PCR, culture or serology was not performed, leishmaniasis cannot be completely ruled out.

Contributing Institution: The University of Texas MD Anderson Cancer Center
Michale E. Keeling Center for Comparative Medicine and Research
http://www.mdanderson.org/education-and-research/departments-programs-and-labs/programs-centers-institutes/michale-e-keeling-center-for-comparative-medicine-and-research/index.html

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CASE III: 12-17590 (JPC 4034294).

Signalment: 6-year-old female polled Hereford cow (*Bos taurus*).

History: Cow is in good body condition on native pasture. She was seen to have an elevated respiratory rate and standing in water. After walking, she had muscle fasciculations in her left hind leg. She then died.

Gross Pathology: Both kidneys exhibit multiple abscesses ranging from 2-10 mm diameter containing greenish caseous material. The right kidney is small and fibrotic. The liver is mildly swollen with a pale khaki coloration on cut surface and is friable. There is ulceration in the distal esophagus. The pericardial surface of the right ventricle contains mild petechiation.

Laboratory Results: Culture (routine): Kidney- moderate pure growth of *Corynebacterium renale*.

Sensitivity:

Sulphafurazole	Sensitive
Trimethoprim	Resistant
Tetracycline	Sensitive
Erythromycin	Sensitive
Penicillin	Resistant
Novobiocin	Sensitive

Hematology:

Parameter	**Value**	**Reference Range/ Units**
RBC	7.06	5.00-8.00 x 10^{12}/ L
PCV	38	23-44 %
HGB	14.6	8.0-15.0 g/dL
MCV	54	44-62 fL
MCH	21 H	14-20 pg
MCHC	38 H	30-35 g/dL
WBC	3.8 L	4.0-12.0 x 10^9 /L
BANDS	0.00	0.00-0.12 x 10^9 /L
NEUT	0.95	0.60-4.00 x 10^9 /L
LYMPH	2.58	2.50-7.50 x 10^9 /L
MONO	0.27	0.03-0.84 x 10^9 / L
EOS	0.00	0.00-2.40 x 10^9 /L
BASO	0.00	0.00-0.20 x 10^9/L
NUCL.RBC	3	/100 WBC

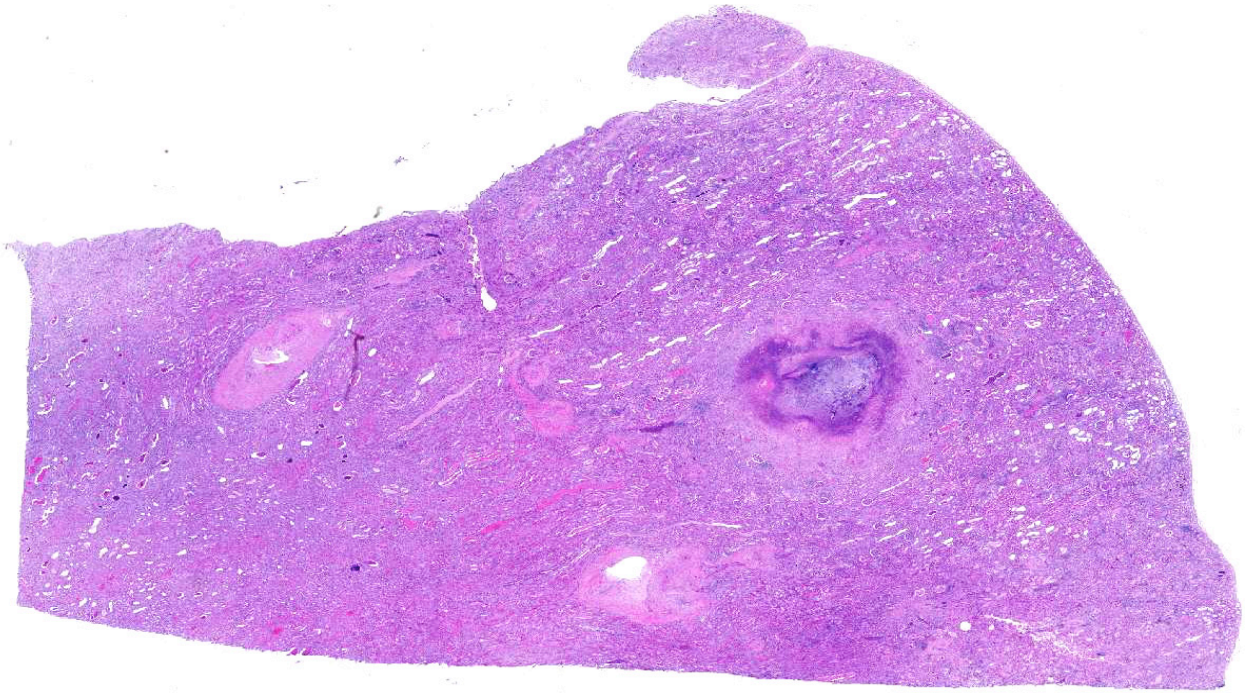
Platelet clumping observed; platelets appear adequate on blood film; Anisocytosis (+); Rouleaux

Biochemistry:

BUN	98.9 H	2.1-10.7 mmol/L
CREA	2550 H	0-186 umol/L
BUN/CREA	0.04	0.00-0.07
PHOS	3.45 H	0.80-2.80 mmol/L
Ca	2.60	2.00-2.75 mmol/L
TP	105.9 H	60.0-85.0 g/L
ALB	36.0	25.0-38.0 g/L
Glob	69.9 H	30.0-45.0 g/L
Alb/Glob	0.5 L	0.7-1.1
AST	998 H	0-120 U/L
GLDH	19	0-30 U/L
GGT	14	0-35 U/L
TBIL	4.0	0.0-24.0 umol/L
CK	3215 H	0-300 U/L
MG	1.05	0.74-1.44 mmol/L
BHB	0.10	0.00-0.80 mmol/L
PROT-RTS	130 H	65-85 g/L
FIBRIN	19 H	3-7 g/L
PR/FI	6 L	15-100

Histopathologic Description: Extending from the renal papilla into the medulla and effacing the normal architecture is irregular, focally extensive necrosis characterized by cellular and nuclear debris. The tubules are ectatic, serpentine and variably lined by vacuolated, attenuated to swollen epithelial cells (degeneration), hypereosinophilic epithelial cells with pyknotic nuclei (necrosis) or hypertrophic crowded epithelial cells with plump vesicular nuclei (regeneration). Tubule lumina often contain deeply eosinophilic proteinaceous material, moderate numbers of degenerate neutrophils and cellular debris. Large numbers of tubules are expanded by anisotropic, refractile, birefringent, pale yellow crystals containing radiating spokes (oxalate crystals) and occasional clusters of coccobacilli. There is moderate expansion of the interstitium with multifocal infiltrates of lymphocytes and plasma cells, mature fibrosis and multifocal loss of tubules and glomeruli. Within necrotic foci are discrete colonies of coccobacili.

Special Stain: Gram-positive coccobacilli noted within necrotic foci.



3-1. Kidney, ox: The section of kidney is diffusely and moderately hypercellular. The center of the cortex contains a focal abscess which is outlined by degenerate neutrophils and cellular debris. (HE 0.63X)

Contributor's Morphologic Diagnosis: Kidney: Pyelonephritis, suppurative, diffuse, severe with tubular ectasia, proteinosis and scant coccobacilli. Kidney: Nephrosis, multifocal, moderate with tubular necrosis, degeneration and regeneration, interstitial fibrosis, and numerous intratubular oxalate crystals.

Contributor's Comment: This case is a classic example of two diseases that occur commonly in cattle; however, what makes this case interesting is that they are occurring concurrently.

The clinical pathology results support the gross and histological findings of severe renal disease, hepatic necrosis (histopathological finding, slide not included), and a significant inflammatory process (elevated immunoglobulins and fibrinogen). The leukogram shows a mild leukopenia, which in this case is likely to be due consumption of leukocytes as part of the inflammatory response. Rouleaux formation is a common finding in hyperglobulinemic or hyperfibrinogenemic states.[7]

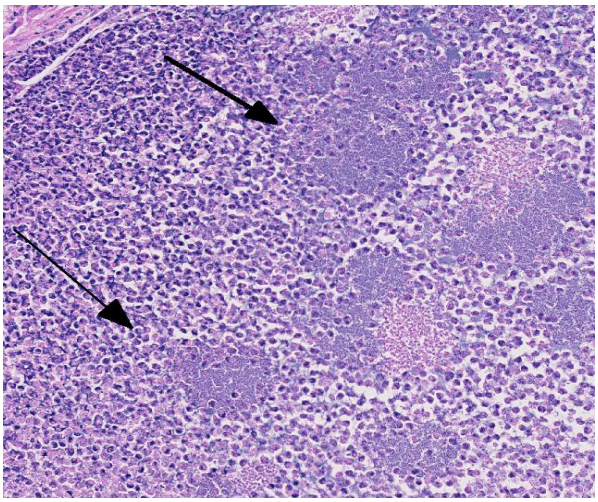
Pyelonephritis is an inflammation of the renal pelvis and renal parenchyma, usually resulting from an ascending infection from the lower urinary tract.[3]

In cattle, *Corynebacterium renale* is a common cause of pyelonephritis.[2] In a survey of clinically affected animals, *C. renale* was the most common bacteria isolated[1] and in a slaughterhouse survey of cattle with gross kidney lesions, it was the third most predominant organism isolated.[6] It is a facultative commensal organism that is commonly isolated from the urinary tracts of healthy cattle.[1]

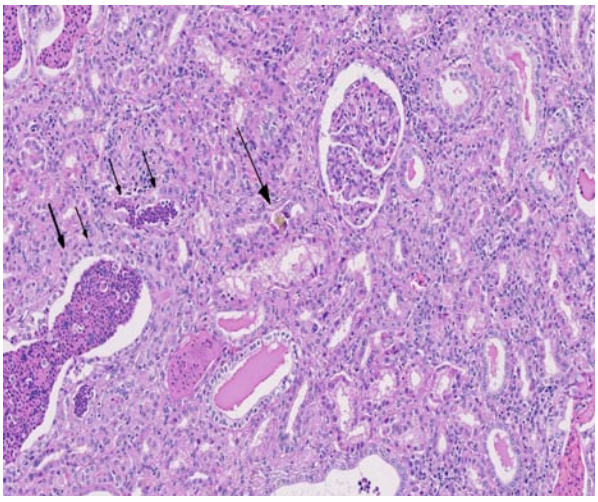
Other common causes of pyelonephritis in cattle include:[6,1]

- *E.coli*
- *Truperella pyogenes* (formerly *Arcanobacter pyogenes)*
- *Corynebacterium cystitides*
- *Corynebacterium pilosum*
- *Streptococcus* spp.
- *Enterococcus faecalis*

Acute pyelonephritis characteristically begins with necrosis and inflammation of the renal crest in an irregular pattern that, as the disease progresses, results in chronic changes where mononuclear cells replace neutrophils and fibrosis eventually predominates. Chronic pyelonephritis occurs more commonly in cattle than acute disease, with acute pyelonephritis often an incidental finding at post mortem.[3]



3-2. Kidney, ox: Even at moderate magnification, large colonies of bacilli are visualized within the center of the abscess. (HE 100X)



3-3. Kidney, ox: Throughout the cortex, tubules are ectatic, and contain variable amounts of protein, degenerate neutrophils, and necrotic epithelial cells (small arrows). Scattered throughout the cortex, there are low numbers of fan-shaped, birefringent oxalate crystals within tubular lumina. (HE 320X)

Vesicoureteral reflux is the most significant mechanism for transporting bacteria from the bladder to the kidney with refluxed urine occasionally being transported to the urinary space of the glomeruli.[3] Bacteria can spread hematogenously to the kidney, but this is a less common mode.

Within the kidney, the medullary region is the most susceptible to infection. This is due to its:

- Relative hypoxia (due to the low haematocrit in the vasa recta)
- Hypertonicity (depressed the phagocytic activity of leukocytes)
- High ammonia concentration (which interferes with the activation of complement).[3]

Several virulence factors make *C. renale* a significant pathogen for cattle. These include:

- Urea hydrolysis, which converts urea to ammonia. Ammonia initiates the inflammatory process and causes suppression of antibacterial defenses through complement inactivation.
- Pilli mediated attachment to the urothelium, which allows for persistence within the lower urinary tract in alkalotic urine. Cattle normally have alkalotic urine due to their herbivorous diet.[8]

Oxalate nephrosis can also be a common finding in ruminants which may manifest as acute or chronic renal disease, abortion or hypocalcaemia without renal damage.[5]

In ruminants, oxalate nephrosis is commonly caused by grazing plants high in soluble oxalate, typically those with greater than 2-2.5% soluble oxalate in dry matter. Many plants of this type contain greater than 10% soluble oxalate. Oxalate is higher in young plants with actively growing leaves. Soils high in nitrogen will also boost the concentration of oxalates.[4] It has been reported that annual deaths in sheep flocks grazing soursob (*Oxalis pes-caprae*) in Australia have had mortalities of 1%, although up to 25% of a flock may be affected.[5]

Naive or hungry cattle are more likely to suffer from oxalate toxicosis. Most oxalate containing plants are not readily eaten under normal grazing conditions and ruminal flora is not adapted to metabolize oxalates.[4]

Oxalate toxicosis occurs by calcium binding of the ingested soluble oxalates in the rumen, blood vessels liver and kidneys and leads to hypocalcaemia and oxalate crystal formation in the nephrons and blood vessels.[5]

The mechanism of injury in the kidney is from both mechanical obstruction of the nephrons and the cytotoxic effects of oxalate metabolites in the renal epithelium.[3]

Plants that are known to cause oxalate nephrosis in Australia and US include:

Scientific Name	Common Name
Halogeton glomerulatus	Halogeton, Barillia
Chenopodium spp.	Fat hen
Sarcobatus vermiculatus	Grease wood
Rumex spp.	Sorrel, dock
Mesembryanthemum spp.	Ice plants
Tetragonia tetragonioides	New Zealand spinach
Trianthema spp.	Black pig weed, Red spinach
Amaranthoretroflexus	Red root amaranth
Beta vulgaris	Beet
Rheum x cultorum	Rhubarb
*Oxalis pes-caprae**	Soursob
Portulaca oleracea *	Pigweed
Acetocella vulgaris	Sheep Sorrel
Atriplex muelleri	Salt Bush
Emex Australia	Spiney emex
Pennisetum ciliare	Buffel grass
*Seratia sphacelata**	Seratia
Acetosa vesicara	Ruby dock
Brassica spp.	

*Denotes common causes of oxalate poisoning in Australia.[5]

Other substances that are known to cause oxalate nephrosis include:

- Fungi (in feedstuffs)
  - *Aspergillus niger*
  - *Aspergillus flavis*
  - Some *Penicillium* spp.
- Ethylene glycol
- Primary hyperoxaluria- a rare genetic disorder that affects Beefmaster cattle
- Pyridoxine (vitamin B6) deficiency
- Excess ascorbic acid (vitamin C)reported in humans and a goat[3]

Although commonly reported, hypocalcaemia associated with oxalate nephrosis is not often seen at out laboratory.

JPC Diagnosis: 1. Kidney: Pyelonephritis, suppurative and necrotizing, chronic, diffuse, severe with large colonies of bacilli.
2. Kidney, tubules: Oxalate crystals, multiple.

Conference Comment: The contributor provides an excellent, thorough review of both pyelonephritis caused by *C. renale* infection and oxalate nephrosis. Microscopically, there are low to moderate numbers of intratubular calcium oxalate crystals with rare foci of minimal granulomatous response; however, these mild histopathologic features in combination with the serum biochemistry results (specifically the lack of hypocalcemia), led conference participants to conclude that oxalate toxicosis did not contribute significantly to the pathogenesis in this case. There was also some debate regarding the composition of the deeply basophilic material often present within necrotic renal tubules. Although most participants initially identified this substance as mineral, the moderator suggested instead that it is composed of aggregates of DNA secondary to widespread necrosis, pointing out its similarity to the microscopic appearance of material seen in acute tumor lysis syndrome, a condition seen in mice. As noted by the contributor, the clinical pathology findings are consistent with severe pyelonephritis due *C. renale*; however, the significant elevations in CK and AST could also result from hemolysis. Additionally, there is some slide variation; not all sections contain renal pelvis and in those that do, medullary tubules are often widely separated by fibrosis (demonstrated with Masson's trichrome). A brief discussion ensued regarding the severity of the medullary fibrosis in this case, with the moderator suggesting that this was within normal limits for ruminants.

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CASE IV: 0386/10 (JPC 4032715).

Signalment: 20-month-old female landrace goat, (*Capra aegagrus hircus)*.

History: This animal was used for teaching physiology to veterinary students at the Swedish University of Agricultural Sciences. The other goats in the herd were all healthy without respiratory symptoms. The goat had been showing signs of dyspnea for 1.5 weeks and was treated with penicillin without improvement. The animal was anorectic, with a temperature of 39.5°C and started to become dehydrated. The goat was euthanized due to increasing dyspnea.

Gross Pathology: The lungs were pale, enlarged and firm. Cut surfaces showed multiple to diffuse solid, poorly circumscribed, pale areas. Regional lymph nodes (mediastinal and tracheo-bronchial) were severely enlarged and pale with well-defined cortex and medulla structures.

The liver had pale severely fibrotic bile ducts, diffusely. Bilateral mild hyperemia of carpal joints was noted as well as mild meningeal hyperemia.

Laboratory Results: An ELISA with antibodies against CAEV (caprine arthritis-encephalitis virus) was performed on a suspension of lung tissue, post mortem. This test was positive for presence of these antibodies.

Histopathologic Description: The lung displays frequent diffuse eosinophilic homogenous fluid and debris in most of the alveolar spaces and bronchiole lumina. The homogenous fluid also contains a small amount of nuclear debris and degenerating granulocytes. The alveolar septal walls are markedly widened due to multiple foci of necrotic debris and moderate diffuse to multifocal infiltration of lymphocytes. There are also moderate multifocal to diffuse, infiltrates of eosinophils within alveolar and bronchiolar septal walls, and perivascularly adjacent to bronchi. A few follicular accumulations of lymphocytes are present in the septal walls.

There is severe type II pneumocyte hyperplasia and hypertrophy, characterized by typical cuboidal cells outlining the septal walls.

Bronchioli are often difficult to separate from alveoli, since the bronchiolar epithelium is hyperplastic and sometimes necrotic. Bronchiolar lumina are also filled with an eosinophilic exudate and nuclear debris, with degenerating eosinophils. The bronchiolar walls show moderate hypertrophy of the smooth muscles.

The interstitial fibrous tissue septa exhibit mild to moderate diffuse infiltration of eosinophils, dilated blood vessels and moderate edema.

There is diffuse mild subpleural edema.

Occasionally within alveoli there are transverse and longitudinal sections of nematode larvae admixed with eosinophilic debris. In some sections rare adult lung worms are present in alveolar lumina. The distinctive pointed tail that is characteristic of a *Protostrongylidae* was not identified; however, only a single transversely cut adult was seen.

In addition, there is eosinophilic and granulomatous lymphadenitis of the mediastinal and tracheobronchial lymph nodes, as well as lymphohistiocytic (subependymal) encephalitis and meningitis.

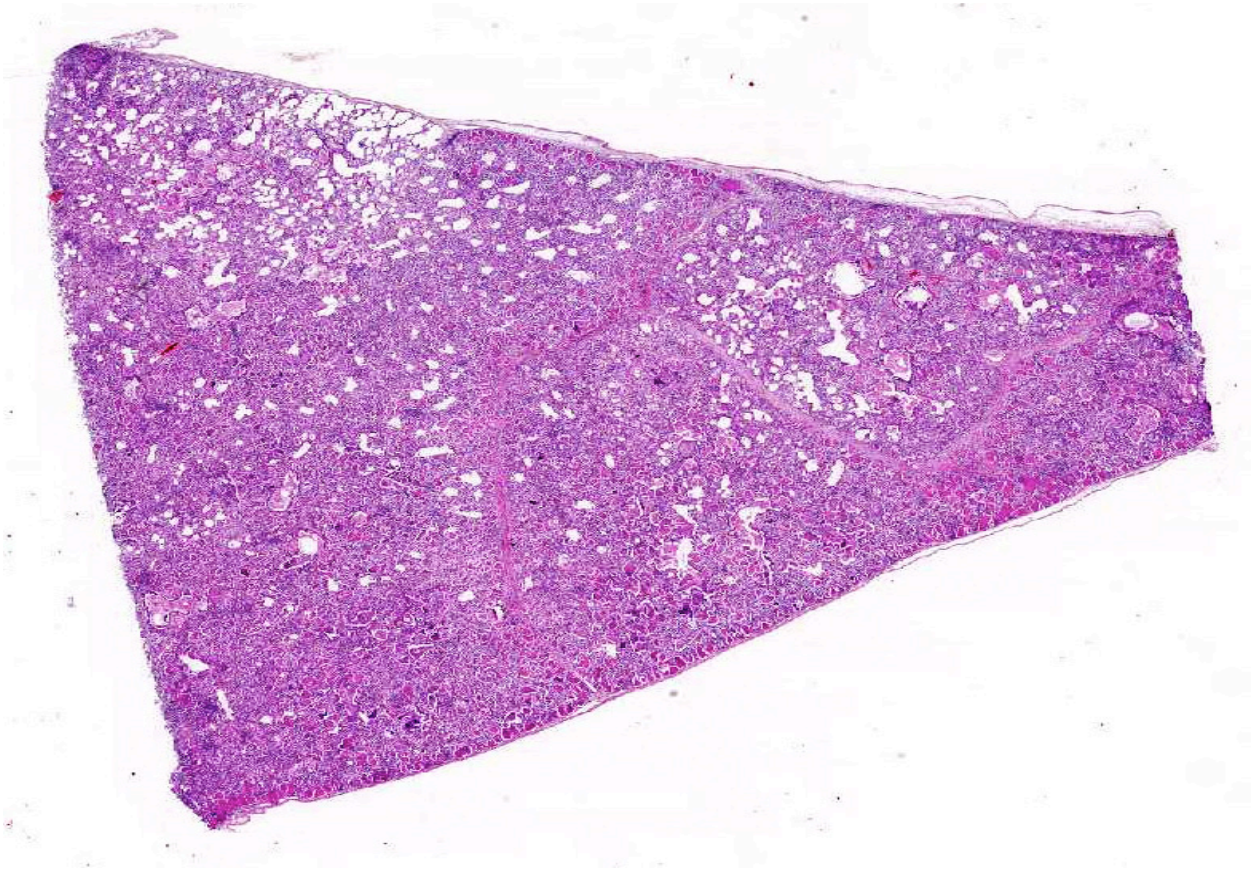
Contributor's Morphologic Diagnosis: Lung: Pneumonia, interstitial, chronic, lymphocytic, severe, diffuse with intra-alveolar eosinophilic fluid and hyperplasia of pneumocytes type II and smooth muscles.

Lung: Pneumonia, interstitial, eosinophilic, moderate, multifocal with few intralesional larvae and adults of small lung worm, (family *Protostrongylidae*).

Etiology: CAEV (caprine arthritis-encephalitis virus).

Contributor's Comment: CAEV (caprine arthritis-encephalitis virus) is a *lentivirus* of the family *Retroviridae*, a member of the small ruminant lentiviruses (SRLVs). CAEV causes arthritis in adult goats, neurologic disease in younger animals and pneumonia that can occur concurrently with arthritis and encephalitis.

The disease is characterized by dyspnea, and the respiratory form is closely related to a small



4-1. Lung, goat: The section of lung is diffusely hypercellular with marked thickening of alveolar septa. (HE 0.63X)

ruminant *lentivirus* infection in sheep called maedi, meaning dyspnea in Icelandic. CAEV and maedi-visna virus have also been found as co-infections in naturally infected goats.[4]

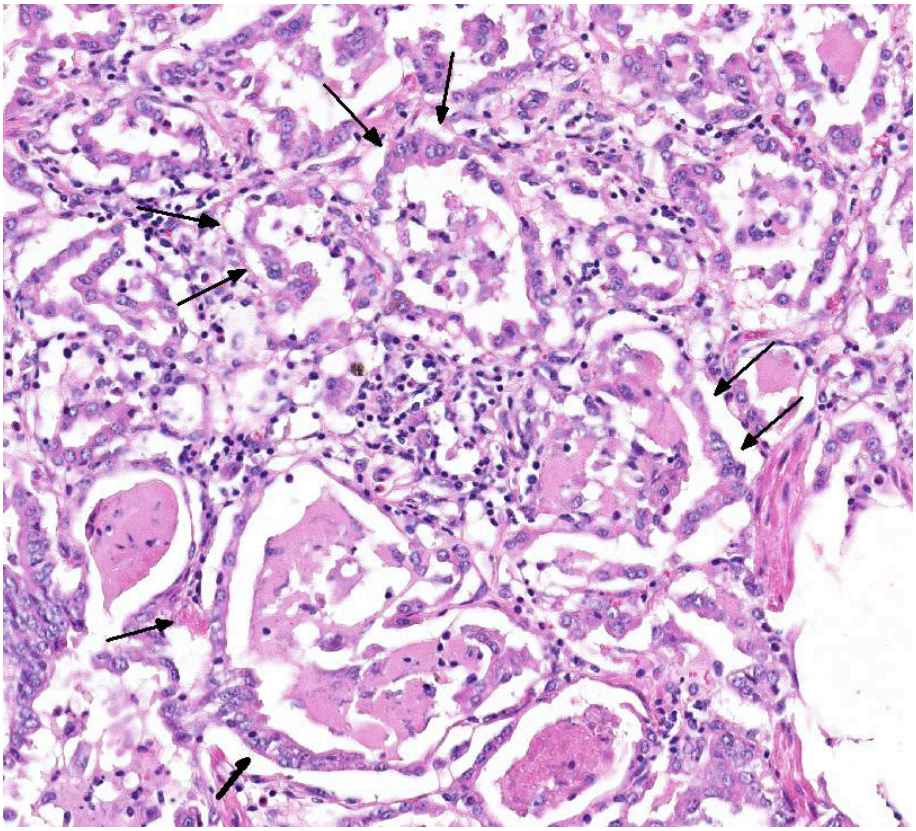
The SRLVs cause chronic multi-systemic inflammatory disease and mammary gland epithelium is often infected. Transmission to young animals is through milk (colostrum) via the intestine, or, less commonly, horizontally via the respiratory tract. Transmission from infected domestic goats through milk to Rocky Mountain goats has recently been described,[3] and in these cases a direct transmission between animals also was reported.

The natural infection of the SRLVs is with virus or virus-infected cells through the mucous membranes such as respiratory or intestinal tracts. The tropism of SRLVs is primarily the monocytes/macrophages and dendritic cells. Infected dendritic cells migrate to lymph nodes and transmit the virus to macrophages. The macrophages are then responsible for systemic infection. Persistent infection can be established in bone marrow cells, and infected monocytes continue the transmission of infection to mainly lung, mammary gland, CNS and joints. The immune activation to the viral antigen causes an inflammatory response with mononuclear cells such as lymphocytes, macrophages and plasma cells, which gives the virus access to more macrophages, continuing the inflammation, hence the multi-systemic disease of SRLVs is immunopathogenic (for review see Blacklaws).[1]

The most common tissues involved in SRLV infection are lung, CNS, mammary gland and joint. Other organs such as kidney, liver and heart can also be involved.

The time between infection and clinical disease is dependent on the strain and amount of the virus as well as the genetic background of the host.

CAEV is characterized by chronic interstitial pneumonia with infiltration of lymphocytes into alveolar septa which are lined by a layer of



4-2. Lung, goat: Alveolar septa are thickened by collagen, marked type II pneumocytes hyperplasia (arrows), and small aggregates of lymphocytes with fewer histiocytes and plasma cells. (HE 200X)

hyperplastic cuboidal type II pneumocytes, and accumulation of eosinophilic fluid within alveoli.

The exudative form of the acute phase is followed by regenerative lesions characterized mainly by hyperplasia as well as hypertrophy of the type II pneumocytes.

The differential diagnosis for interstitial pneumonia is extensive;[2] however, the typical type II reaction together with accumulation of eosinophilic fluid within alveoli and diffuse infiltration of lymphocytes is typical for CAEV infection. This pneumonia, together with multisystemic involvement including arthritis and encephalitis, further strengthens the diagnosis, which was confirmed by immunological testing.

Muellerius capillaris is a common small (12 -24 mm adults) lungworm of goats and sheep. Infection with this nematode is often characterized by nodular inflammation with eosinophils and giant cells; however, the inflammation depends on the amount of larvae/ adults present in the alveoli. Eggs are rapidly hatched and first-stage larvae are found in alveolar spaces. These are coughed up, swallowed and eventually found in the feces. Intermediate hosts are snails and slugs and the life cycle of the nematode is completed when the goat digests the intermediate host. The larvae will migrate via lymphatics to the lungs and adults can be seen in the alveolar spaces.

Subpleural nodules are a characteristic finding in heavy worm infections, but this was not seen in the present case.

Another member of the family *Protostrongylidae* is *Protostrongylus rufescens*, which measure 16-35 mm. These small lung worms have a similar life cycle to *Muelleris capillaris* and also cause inflammation in the lungs with pulmonary nodules.

The few larvae/adults found in the present case could not be classified on histological slides as *Muellerius* or *Protostrongylus*.

The clinical symptoms and severity of the inflammatory reaction depend on the number of invading worms, the stage of the larvae and the immune status of the animal. The severity of the pneumonia in this case does not correlate with the animal's low parasite burden; thus CAEV infection is a much more likely diagnosis.

JPC Diagnosis: 1. Lung: Pneumonia, interstitial, lymphohistiocytic, chronic, diffuse, severe, with marked type II pneumocyte hyperplasia and alveolar proteinosis.
2. Lung, alveoli: Trichostrongyle larvae, multiple.

Conference Comment: This case is an excellent example of a classic entity, and the contributor provides a thorough review of the disease

pathogenesis, clinical signs and gross and microscopic lesions. Readers are urged to review WSC 2011-2012, Conference 7, case 2 for further discussion of CAEV.

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