American Society for Veterinary Clinical Pathology
CASE DISCUSSION
CASE 1

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SIGNALMENT: Group of 5-month-old Holstein heifers

HISTORY AND CLINICAL FINDINGS:
Sudden death occurred in 6 of 36 calves from a group pen in southeast Georgia. Seven other calves were in various states of recumbency and exhibiting multiple neurologic symptoms. All remaining calves were ambulatory but also showed neurologic signs including ataxia, excessive licking behavior, head pressing, or head tremors. An employee of the farm reportedly noticed the water trough was not working approximately 24 hours before the first death and had repaired it within 2 hours of finding the issue. Samples were collected roughly 5 hours after the first death occurred.

LABORATORY DATA:

Chemistry results:

Table 1. Results of electrolyte levels in serum of 4 heifers via NOVA panel performed at UGA.

TEST	UNITS	Heifer #1	Heifer #2	Heifer #3	Heifer #4
Sodium	mmol/L	136.4	132	133.4	134.1
Potassium	mmol/L	5.19	5.57	7.02	5.87
Chloride	mmol/L	101.0	94.1	95.7	99.4
Ionized Ca	mmol/L	1.08	1.06	0.92	1.12
Ionized Magnesium	mmol/L	0.46	0.43	0.42	0.50
Glucose	mg/dl	39	119	62	49

Table 2. Results of electrolyte levels in CSF of 3 heifers performed at Michigan State University Veterinary Diagnostic Lab.

TEST	UNITS	Heifer A	Heifer B	Heifer C
Sodium	mmol/L	134	127	134
Potassium	mmol/L	15.4	25.8	11.5
Chloride	mmol/L	109	105	113

Table 3. Results of electrolyte levels in ocular fluid of 3 heifers performed at Michigan State University Diagnostic Lab.

TEST	UNITS	Heifer A	Heifer B	Heifer C
Sodium	mmol/L	135	138	135
Potassium	mmol/L	10.2	11.3	6.6
Chloride	mmol/L	104	110	107

QUESTIONS:

1) What is the diagnostic threshold for salt toxicity in cattle?

   A. 130 mmol/L
   B. 140 mmol/L
   C. 150 mmol/L
   D. 160 mmol/L

2) What protective/compensatory mechanisms normally take place in the body under conditions of hypernatremia?

INTERPRETATION: Suspect salt toxicity (water deprivation→sodium ion intoxication)

ADDITIONAL FINDINGS:

Histopathology of brain for heifer A:
Cerebrum: There are multifocal hemorrhages in the cerebral cortex and white matter. The corona radiata is finely vacuolated (possible edema). Perineuronal and perivascular clear spaces (most consistent with edema) are present in the cortex where there are bands of shrunken neurons with little cytoplasm (necrosis) that are close together as if the neuropil has collapsed (laminar necrosis).

Cerebellum: Multifocally scattered within the cerebellar white matter are aggregates of extravasated erythrocytes (hemorrhage) and increased clear space (edema). Perivascular tissue surrounding all caliber vessels is mildly to markedly displaced by increased circumferential clear space (possible edema or artifact). There are also hemorrhages in the molecular and internal granule cell layers of what is thought to be the vermis (suspect vermis herniation). The meninges in this area are severely congested with hemorrhage and there is a mild infiltration of neutrophils admixed with a few eosinophils. One cerebellar nucleus along this area has necrotic neurons.

Histopathologic Diagnoses:
Cerebrum: Mild, multifocal acute hemorrhages; white matter edema; perineuronal and perivascular cortical edema; and laminar cortical neuronal necrosis
Cerebellum: Mild multifocal neuroparenchymal hemorrhage; white matter edema; cerebellar nucleus with neuronal necrosis; mild, acute neutrophilic and eosinophilic meningitis with hemorrhage

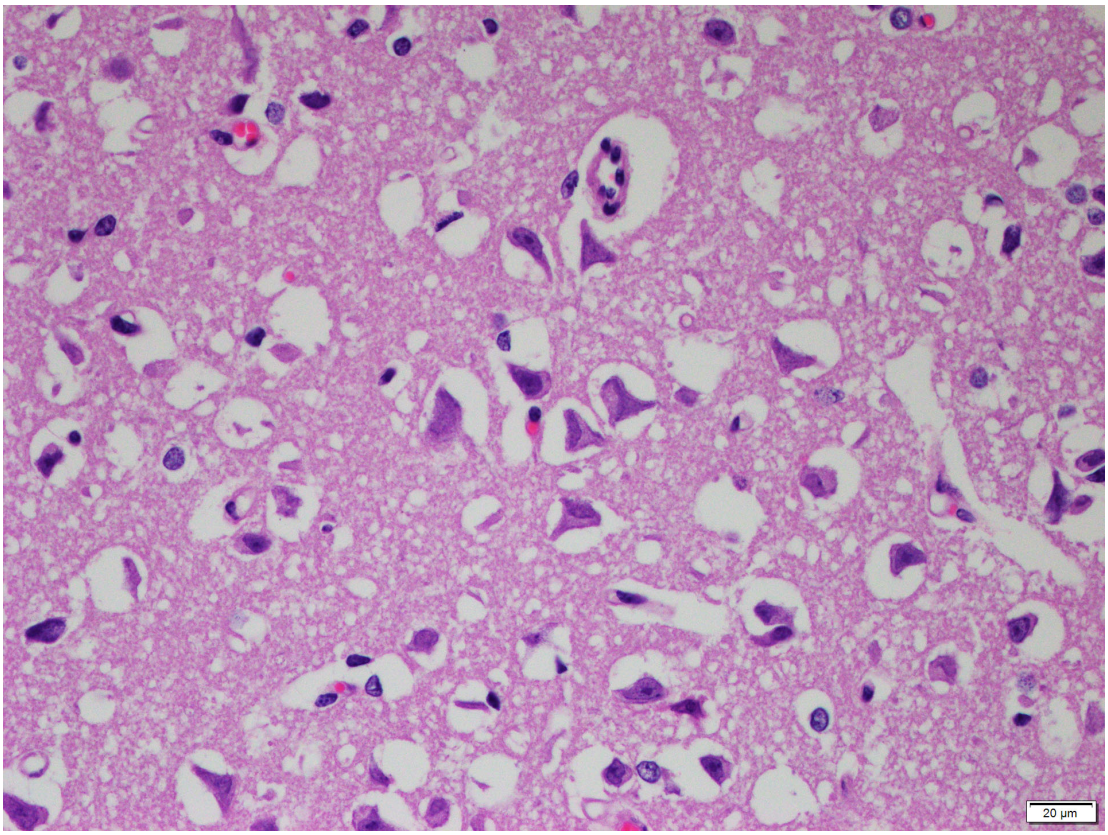

Figure 1. Histopathology of the cerebrum (gray matter). Hematoxylin & eosin, 40X. Diffusely, there is perineuronal and perivascular clear space (most consistent with edema). Many neurons in the cortex are shrunken and close together and many have eosinophilic cytoplasm and nuclear changes consistent with necrosis.

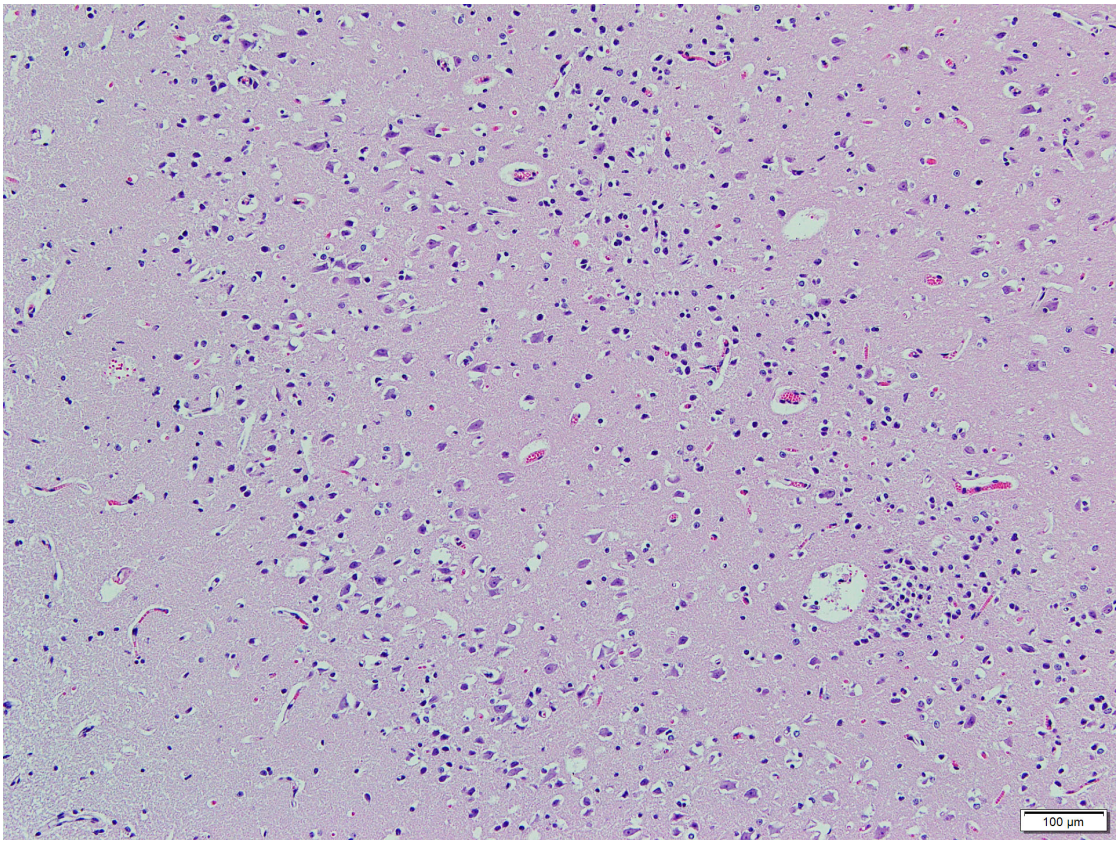

Figure 2. Histopathology of the cerebrum (gray matter). Hematoxylin & eosin,10X. Laminar cortical necrosis is evidenced by bands of small nuclei next to the more normal bands of neurons.

Heifers A-C all had similar histopathologic features in the brain. Histopathology was not submitted for heifers 1-4.

ANSWERS TO QUESTIONS:

1) D
2) Answered as part of discussion.

DISCUSSION:

Salt (sodium chloride) is an essential nutrient to all animals.[1] It is necessary for maintaining transport systems in the body as well as regulating pH, temperature, and osmolarity, among other functions. It usually is well-tolerated at high concentrations if there is concurrent fresh water access.[1] An excess of dietary salt and/or inadequate water intake can both lead to salt poisoning; the latter is considered more common.[1] Hypernatremia caused by the gain of sodium causes expansion of the extracellular fluid (ECF) with a relative dehydration of intracellular fluid as an attempt to normalize plasma osmolality.[2] Hypernatremia caused by water restriction causes absolute dehydration and a reduction in total body water.[2] It is important to note that "salt poisoning" caused by water restriction leads to an indirect sodium toxicosis, with subsequent water toxicity on rehydration (this is actually what leads to death in this case). For simplicity, this disease will often herein be referred to as simply "salt toxicity."

Salt is normally present in the diet at levels of 0.5-1% dry matter for bovine and roughly 90% is absorbed by the gastrointestinal tract.[1] Salt requirements increase with lactation, exertion, and environmental temperature and the corresponding water requirements will also increase.[1] The acute toxic dose of sodium chloride is roughly 2.2 g/kg body weight in bovine, as well as swine.[1] Some cattle are given high-salt diets to limit feed intake, and many have access to salt blocks or mineral mixes in addition to the diet.[1] Improperly mixed diets, as well as exposing animals previously salt-deprived to high-salt feeds, can also lead to excessive salt intake.[1,] Sources of salt other than the diet include high-saline ground water, seawater, and whey.[1] A herd problem of salt toxicity has been seen in dairy calves that were given milk replacer mixed with high salinity water.[2]

Excessive salt can be irritating to gastrointestinal mucosa, causing anorexia, vomiting, and/or diarrhea.[1] Ingesting excess sodium can cause hypernatremia leading to clinical signs in 1-2 days. However, it is more common that water intake is reduced or restricted, leading to an indirect sodium toxicosis.[1] Causes of restricted water intake include frozen pipes, mechanical failure, overcrowding of animals, unpalatable water, and owner neglect.[1]

Sodium is the main cation that regulates osmotic balance in the ECF.[1] Its concentration, as well as serum osmolarity, is maintained by thirst, renal retention/absorption, and antidiuretic hormone. Normal levels of sodium for adult bovine serum are between 132-152 mmol/L.[1] An increase in salt intake leads to hypernatremia which then distributes throughout the body.[1] Normally, the body takes action to correct for this increase in sodium and subsequent increase in osmolarity. Osmoreceptors in the hypothalamus sense the increase in osmolarity of the ECF and stimulate thirst. Antidiuretic hormone is released by the posterior pituitary gland, causing the kidneys to retain more water. However, if water is not available or osmolar changes are too dramatic, these mechanisms may not be sufficient to maintain homeostasis.[1]

As sodium levels increase in serum, water will follow along the osmotic gradient, flowing out of the interstitium and into the vasculature, while intracellular fluid flows into the ECF.[1] Sodium can also diffuse across the blood-brain barrier, increasing the sodium concentration in cerebrospinal fluid.[1] In order to prevent water loss to the ECF and subsequent cell shrinkage, neurons will increase their intracellular osmolarity.[1] If this protective mechanism fails, then cell shrinkage occurs, leading to disruptions in blood supply and the potential for hemorrhages in the brain.[1] If there continues to be increased concentration of sodium in neurons, glycolysis is inhibited.[1] It is important to note that sodium can passively diffuse into the brain, but requires active transport to be moved out.[1] Therefore, response to a rapid decrease in serum sodium (which may be seen with sudden excessive consumption of water) will be delayed and water may move into the brain, causing cell swelling and cerebral edema.[1] In cases of chronic hypernatremia (at least 48-72 hours), the brain will have produced idiogenic osmoles, which are more osmotically active and take 42-78 hours to decrease in concentration once the hypernatremia is corrected.[1] Therefore,

a rapid decrease in serum sodium after chronic hypernatremia can, as in an acute situation, lead to water moving into the brain along an osmotic gradient that leads to cerebral edema.[1]

With acute salt poisoning, cattle may show signs of gastroenteritis, weakness, ataxia, and dehydration.[1] They may also appear blind and develop seizure-like activity. Eventually, affected cattle may be found in lateral recumbency with neurologic signs such as paddling, and may die within 24 hours.[1] If animals do recover, they may exhibit mild signs such as non-painful knuckling of their feet.[1]

Diagnosing salt toxicity can be difficult and requires components of both a thorough history and certain pathologic findings. Necropsies performed in these cases may reveal gastric irritation, and content of the gastrointestinal tract may be very dry.[1] More notably, lesions may be limited to the brain and can include cerebral edema (as in the heifers in this case) and meningitis.[1] Eosinophilic perivascular cuffs are considered common in swine but are also sometimes noted in cattle.[1] Other potential lesions include muscular edema and hydropericardium. In very acute cases, there may be no identifiable lesions.

A brain sodium level above 2000 ppm is considered diagnostic for salt toxicity in both cattle and swine (not available for this case).[1] Serum taken from a living animal generally has sodium levels markedly increased over the reference interval.[1] CSF samples are also expected to have high sodium levels but these samples can be difficult to obtain and are often contaminated by blood.[3] Generally, CSF and serum with sodium levels >160 mEq/L are indicative of salt toxicity.[1] Aqueous fluid is considered a good postmortem specimen because the sodium does not change much with postmortem autolysis.[3] Another potential diagnostic aid for salt toxicity is salt concentration in the rumen; 0.5% salt has been suggested as diagnostic.[3] However, this value will vary with rehydration status and based on the source of excess salt.[3]

Although the numerical data for this case do not perfectly fit the high levels expected, the history, clinical signs and histopathologic findings together are most consistent with water restriction and indirect salt toxicity. The concentrations of electrolytes in extracellular fluid return to normal levels very quickly after rehydration, which may explain why the levels obtained in these patients are lower than expected. Changes seen in the cortex were mild but consistent with early polioencephalomalacia and considered consistent with water intoxication. However, the histopathologic changes are nonspecific and can be seen with other diseases such as lead poisoning, sulfur toxicosis, and hypoxia. This was presumed to be a case of water restriction that led to indirect salt toxicity and subsequent water toxicity (upon rehydration) causing cerebral edema and, ultimately, death. This case describes an uncommon entity and leads to an interesting discussion about sodium regulation and the criteria used/needed to diagnose salt toxicity.

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1. Thompson, LJ. Sodium Chloride (Salt). In: Gupta, RC. *Veterinary Toxicology: Basic and Clinical Principles*. 3rd ed. 479-482.
2. Ollivett, TL and McGuirk SM. Salt poisoning as a cause of morbidity and mortality in neonatal dairy calves. *J Vet Intern Med* 2013; 27: 592-595.
3. Osweiler, GD, Carr, TF, Sanderson TP, et al. Water deprivation-sodium ion toxicosis in cattle. *J Vet Diagn Invest* 1995 *(*7): 583-585.

American Society for Veterinary Clinical Pathology
CASE DISCUSSION
CASE 2

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SIGNALMENT: 11-year-old castrated male Boxer

HISTORY AND CLINICAL FINDINGS: The patient was presented to the Michigan State University Veterinary Medical Center (MSU VMC) with a complaint of acute left forelimb lameness that began the previous evening and was associated with trembling and shaking. Prior to this, he had been eating and drinking well, but the owner reported that the dog had been urinating a lot and had incontinence for about three years. Six months prior, the dog was hospitalized at the MSU VMC for 48 hours following repair of a bladder rupture that occurred secondary to obstructive urolithiasis. Two uroliths were removed and analyzed by the Minnesota Urolith Center; one was 100% struvite, and one was 25% calcium oxalate monohydrate and 75% calcium oxalate dihydrate. At that time, an unexplained mildly regenerative, macrocytic, hypochromic anemia was noted. Two months later, a grade I soft tissue sarcoma was removed from the left elbow; histologic evaluation indicated incomplete margins. The dog was currently receiving 14 mg/kg amoxicillin-clavulanate for a ruptured cyst on his right rear leg, and he was being fed a prescription diet (Royal Canin Urinary SO).

On physical exam, the patient appeared alert and responsive with a grade 3/5 left forelimb lameness. Shoulder manipulation yielded a painful response. A firm mass palpated cranial and medial to the left scapula measured 3 cm in diameter. Orthopedic abnormalities were not detected. Two further subcutaneous masses, previously diagnosed cytologically as follicular cysts, were present on the right thigh. The remainder of the exam was within limits of health.

Computed tomography (CT) of the neck and thorax before and after intravenous administration of 600 mg/kg iopamidol (300 mg/ml) revealed moderately enlarged superficial cervical lymph nodes. A small amount of fluid centered at the level of the shoulder and cervical lymph nodes was suspected to be cellulitis or edema. Multiple, small irregularly shaped mineral foci were present bilaterally in the lung lobes and were interpreted as incidental pulmonary osteomas.

Due to the patient's history of possible incomplete excision of a left elbow sarcoma, metastasis to the cervical lymph nodes was suspected. Surgery to remove the lymph nodes (and right hind limb cysts) was scheduled for the following day. Routine preoperative blood and urine samples were collected. The results are provided below.

LABORATORY DATA:

Hemogram results (ADVIA 2120, Siemens)

TEST	RESULT		REF. INT.
Hct, spun	**31**	%	43 - 58
Hct, instrument	**33**	%	43 - 59
pTP (refraction)	7.1	g/dL	6.5 - 8.1
WBC (corrected)	**15.8**	$\times 10^3/\mu L$	4.6 - 10.7
WBC (instrument)	**16.1**	$\times 10^3/\mu L$	4.6 - 10.7
RBC	**4.2**	$\times 10^6/\mu L$	6.3 - 8.2
Hgb	**10.4**	g/dL	14.8 - 20.5
MCV	**78**	fL	66 - 75
MCH	25	pg	22 - 26
MCHC	**31**	g/dL	33 - 36
CHCM	**30**	g/dL	33 - 35
RDW	**15**	%	12 - 14
Platelets	312	$\times 10^3/\mu L$	180 - 366
MPV	10.0	fL	8.2 - 13.6
Neutrophils, segs	**14.4**	$\times 10^3/\mu L$	2.7 - 7.8
Neutrophils, bands	**0.2**	$\times 10^3/\mu L$	0.0 - 0.1
Lymphocytes	**0.0**	$\times 10^3/\mu L$	0.6 - 5.0
Monocytes	**1.3**	$\times 10^3/\mu L$	0.1 - 0.8
Eosinophils	0.0	$\times 10^3/\mu L$	0.0 - 1.3
Basophils	0.0	$\times 10^3/\mu L$	0.0 - 0.1
nRBCs	**2**	/100 WBC	0 - 1

Microscopy: Mild increases in polychromatophils, acanthocytes, elliptocytes, Howell-Jolly bodies, and presumed siderocytes (RBCs with clusters of fine basophilic inclusions)

Venous blood gas analyzer results (Stat Profile pHOx, Nova)

TEST	RESULTS		REF. INT.
pH at 37 °C	**7.13**		7.36 - 7.44
pCO_2 at 37 °C	30.9	mmHg	24.5 - 41.0
pO_2 at 37 °C	**60.5**	mmHg	33.3 - 59.5
SO_2	80.5	%	55.5 - 90.3
Bicarbonate	**10.3**	mmol/L	16.0 - 25.0
BE-Blood	**-17.2**	mmol/L	-5.1 - 1.9
Free calcium	**5.9**	mg/dL	3.4 - 5.3
Normalized fCa	**5.1**	mg/dL	3.6 - 5.4
Free magnesium	1.3	mg/dL	-
Lactate	0.8	mmol/L	0.2 - 3.3

Serum chemistry results (AU680, Beckman Coulter)

TEST	RESULTS		REF. INT.
Urea nitrogen	**11**	mg/dL	12 - 27
Creatinine	1.0	mg/dL	0.6 - 1.5
Sodium	147	mmol/L	139 - 151
Potassium	4.2	mmol/L	3.9 - 5.1
Chloride	**129**	mmol/L	106 - 115
Bicarbonate	**10**	mmol/L	17-27
Anion gap	12	mmol/L	12 - 22
Calcium	**9.0**	mg/dL	9.5 - 10.8
Phosphorus	5.3	mg/dL	2.7 - 5.4
Magnesium	**1.5**	mg/dL	1.7 - 2.3
Iron	**43**	µg/dL	109 - 250
Total protein	5.6	g/dL	5.4 - 6.7
Albumin	**2.4**	g/dL	2.8 - 3.6
Globulins	3.2	g/dL	2.3 - 3.7
Glucose	113	mg/dL	81 - 118
Amylase	**1077**	U/L	266 - 970
ALP	48	U/L	10 - 92
Total bilirubin	0.1	mg/dL	0.1 - 0.3
ALT	35	U/L	21 - 68
AST	19	U/L	16 - 41
CK	86	U/L	51 - 169
Cholesterol	323	mg/dL	126 - 325

Urinalysis results, voided

TEST	RESULTS	TEST	RESULTS
Color / clarity	Pale yellow / Clear	**Ketones**	Negative
Specific gravity	1.008	**Bilirubin**	Negative
pH	7.2	**Glucose**	Negative
Protein	Negative	**WBC**	0-1 /hpf
Heme	Negative	**Epithelial cells**	>40 /lpf

No RBCs, casts, bacteria, or crystals seen
Urine culture and sensitivity: no bacterial growth

QUESTIONS:

1. What is the likely cause of acidemia in this dog, and what further supportive or confirmatory testing could be done?

2. What differentials should be considered to explain the increased free calcium concentration in this dog?

INTERPRETATION/DIAGNOSIS:
Distal renal tubular acidosis (DRTA) characterized by hyperchloremic non–anion-gap metabolic acidosis with inappropriate alkalinuria and accompanied by persistent mild free hypercalcemia and a mildly to nonregenerative hypo- to normochromic, macrocytic, mild anemia of unknown pathogenesis

ADDITIONAL FINDINGS:
There was no prior history of nephrotoxic drugs or novel diets. Serum microscopic agglutination titers for *Leptospira interrogans* serovars were negative**.**

Selected results from a trace nutrient panel (serum):

TEST	RESULT	REF. INT.
Copper	0.59 µg/mL	0.5-0.8
Zinc	0.52 µg/mL	0.8-1.8

Toxic elements (heavy metals) screen (blood): All results were within expected intervals.

Urine Metabolic Screening (PennGen, University of Pennsylvania): No evidence of a genetic metabolic disorder was found based on screening for amino acids, organic acids, carbohydrates, cysteine, ketones, glucose, and mucopolysaccharides.

Histopathology:
- **Cervical lymph node, and surrounding tissue:** Severe pyogranulomatous to necrotizing lymphadenitis and cellulitis
- **Leg masses:** 1) fibroadnexal dysplasia with follicular rupture and secondary pyogranulomatous dermatitis, and 2) follicular cyst and adjacent comedones

Culture of cervical lymph node region: No bacterial growth, anaerobic or aerobic

OUTCOME/FOLLOW-UP:
The patient was managed successfully for 2.5 years before he was lost to follow up. Cellulitis and lameness had resolved by two weeks post-surgery. Therapy varied over time but included sodium bicarbonate (1.2-4.6 mEq/kg/day) and potassium citrate (54 mg/kg/day) for alkalinization, phenylpropanolamine (3.75 mg/kg/day) for urinary incontinence, and alendronate (1 mg/kg/day) for the free hypercalcemia in an attempt to reduce bone resorption. Progressively aggressive alkalinization therapy was associated with a progressive increase in blood pH over 24 weeks, at which time it almost reached the lower reference limit while urine pH dipped below 7.0 (Figure 1). Two weeks later, the dog developed a urinary tract infection and a reduction in blood pH, both of which resolved with antibiotic therapy. At this time, his diet was changed from Royal Canin Urinary SO to Hill's U/D, a diet low in sodium and protein and potentially helpful for reducing sodium diuresis and polydipsia. Owners reported a marked reduction in the severity of incontinence after change to Hill's U/D, and phenylpropanolamine was ultimately discontinued. Despite continued alkalinization, he remained mildly acidemic.

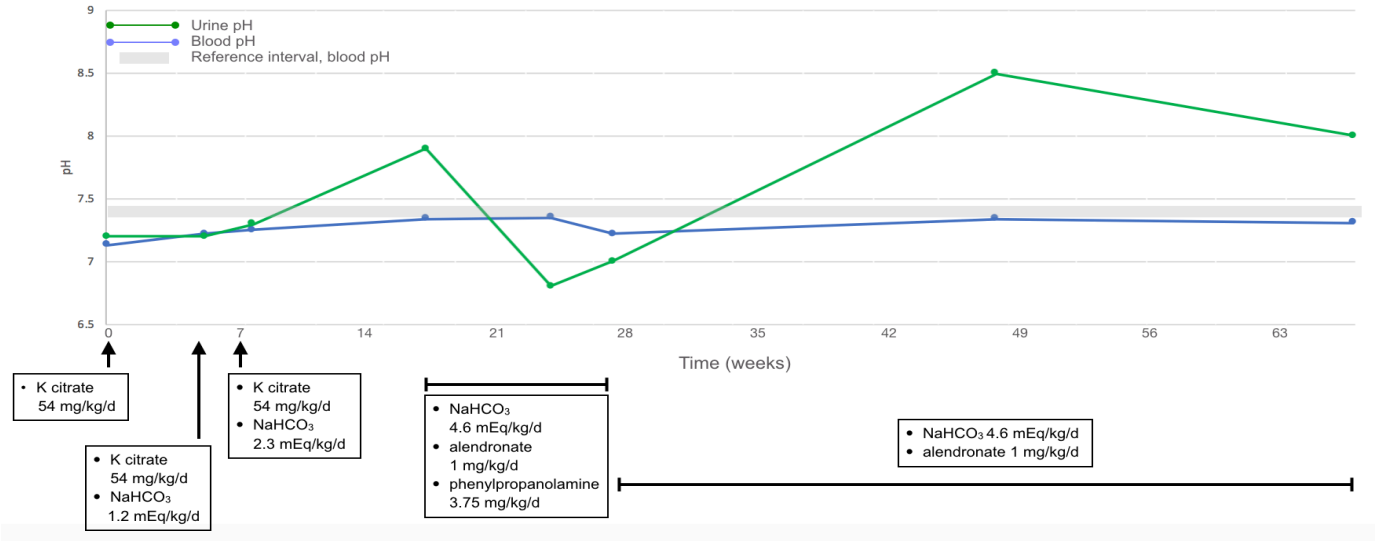

Figure 1. Urine and whole blood pH in relation to treatments over time.

Because of concerns about the initial free hypercalcemia, which was still present (5.9 mg/dL; RI: 3.4-5.3) when checked 2 weeks later, parathyroid hormone (PTH), and PTHrP testing was done to assess for hyperparathyroidism and hypercalcemia of malignancy. This revealed persistent, mild, normalized free hypercalcemia without evidence of mediation by PTH or PTHrP. Ultrasonography of the cervical region 12 weeks after initial presentation revealed bilateral diffuse mottling of the thyroid glands, with numerous hypoechoic patches throughout. The cranial poles of the right and left thyroid glands each contained a hypoechogenic nodule (3 mm and 2.5 mm in diameter, respectively) interpreted as parathyroid hyperplasia, parathyroid adenomas, or thyroid follicular cysts. These lesions were not further explored by biopsy and histologic evaluation.

PTH, PTHrP panel (serum), 2 wk and 12 wk after presentation:*

TEST	RESULTS		REF. INT.
	Week 2	Week 12	
PTH	1.10 pmol/L	1.00 pmol/L	0.50 - 5.80
fCa**	**1.52** mmol/L	**1.49** mmol/L	1.25 - 1.45
PTHrP	0.0 pmol/L	Not done	0.0 - 1.0

* MSU Veterinary Diagnostic Laboratory

** Value normalized to pH 7.4 based on an empirical formula involving the sample [fCa] and pH measured after acidification with HCl (routinely added to counter in vitro increases in pH that can move values outside the analyzer's analytical range)

For the following 14 months, the whole blood nonnormalized [fCa] was persistently 5.6-6.1 mmol/L (RI: 3.4-5.3), and the phosphorus was 4-5.3 mg/dL (RI: 2.7-5.4), not supportive of PTH- or PTHrP-mediated hypercalcemia. Long-term monitoring revealed no evidence of glomerular or proximal tubular disease, hepatic disease, autoimmune disease, chronic urinary infections, or a recurrent neoplasm. Anemia persisted but was generally mild with inconsistent macrocytosis that was initially concurrent with reticulocytosis but later was not (by week 32).

Hemogram results (ADVIA 2120, Siemens)

TEST	RESULT					UNITS	REF. INT.
	0 wk	8 wk	28 wk	32 wk	52 wk		
Hct, spun	**31**	43	**32**	**39**	**40**	%	43-58
RBC	4.2	5.9	4.4	11	13.5	$\times 10^6/\mu L$	6.3-8.2
Hgb	**10.4**	**14**	**11**	**13.5**	**14.1**	g/dL	14.8-20.5
MCV	**78**	**76**	73	**77**	71	fL	66-75
MCH	25	24	25	25	24	pg	22-26
MCHC	**31**	**31**	34	33	34	g/dL	33-36
CHCM	**30**	**31**	**32**	**31**	**32**	g/dL	33-35
Reticulocytes	**284**	**113**		12.4		$\times 10^3/\mu L$	12-76

Microscopy: Mild increases in polychromatophils, acanthocytes, elliptocytes, Howell-Jolly bodies, and presumed siderocytes (RBCs with clusters of fine basophilic inclusions) were consistent findings until 28 weeks post presentation; then, increased elliptocytes, acanthocytes, and presumed siderocytes persisted without polychromatophils or Howell-Jolly Bodies

ANSWERS TO QUESTIONS:

1. What is the likely cause of acidemia in this dog, and what further supportive or confirmatory testing could be done?

   DRTA, as there is a hyperchloremic, non–anion-gap metabolic acidosis with alkalinuria and no evidence of proximal renal tubular damage or other causes of a non–anion-gap metabolic acidosis, which would be associated with a known therapeutic agent, signs of gastrointestinal disease, and/or compensatory aciduria.

   Other tests for DRTA
   Although not necessary in this case, the following tests could be useful to support defects in distal renal tubular H^+ secretion.
   - Urine $[NH_4^+]$: With a validated assay and reference values, **reduced urine $[NH_4^+]$** is expected in DRTA because decreased distal H^+ secretion impairs trapping of ammonia (NH_3) in the collecting duct lumen. In patients with suspected incomplete DRTA and therefore without inappropriately high urine pH, NH_4^+ loading with ammonium chloride can be used to demonstrate a failure to properly acidify the urine and to excrete NH_4^+.
   - Urine anion gap: Given appropriate urine electrolyte methods and reference values, urine anion gap ($[Na^+] + [K^+] – [Cl^-]$) can be used as an indirect index of urinary NH_4^+ excretion. Because NH_4^+ excretion is typically accompanied by Cl^- excretion, decreased NH_4^+ excretion is expected to cause an **increased urine anion gap** (increased unmeasured cations). With ammonium chloride loading, urine anion gap should decrease and become negative, but it will remain high in DRTA. Substantial alterations in other unmeasured cations or anions (e.g., bicarbonate, ketoanions) can alter results, as can hypovolemia and renal failure.
   - Urine citrate: Acidemia decreases the luminal pH of the proximal tubule, thus increasing proximal tubular resorption of citrate leading to **hypocitraturia** (this does not occur with proximal tubular acidosis, despite acidemia, because of a more alkaline luminal pH from defective proximal HCO_3^- conservation).
   - Urine pCO_2: In the presence of normal serum HCO_3^-, pCO_2 should reflect the function of the H^+ pump leading to **decreased** values with DRTA. Similarly, an increased urine to blood pCO_2 gradient occurs in DRTA with bicarbonate loading.

2. What differentials should be considered to explain the increased free calcium concentration?
    - Metabolic acidosis - see Discussion (López, et al., 2004)
    - Increased Ca^{2+} mobilization from bone or absorption in the intestine:
        - Increased PTH or PTHrP activity – laboratory data not supportive
        - Hypervitaminosis D (exogenous or endogenous sources) – not specifically tested, but no suggestive clinical findings or progression over 2.5 yr
        - Neoplasms in bone – unlikely given no progression over 2.5 yr
    - Decreased urinary excretion of Ca^{2+}:
        - Renal failure – the dog was never in renal failure
        - Hypoadrenocorticism – there were no supportive clinical or laboratory findings
        - Administration of thiazide diuretics – not administered

DISCUSSION:

DRTA (type I renal tubular acidosis) is an uncommon diagnosis in dogs, with only a few published reports (Martinez & Hostutler, 2014; Shearer, et al., 2009; Polzin, et al., 1986; Cook, et al., 2011). The causes and clinical features of human DRTA are better understood, but whatever the underlying cause, DRTA results from a failure to secrete H^+ from type A intercalated cells in the connecting tubule and collecting duct of the distal nephron (Alexander & Bitzan, 2019). This leads to the characteristic biochemical features: hyperchloremic, non–anion-gap metabolic acidosis with normokalemia or hypokalemia, decreased renal ammonium excretion, hypercalciuria, and hypocitraturia. The disorder is progressive and can lead to severe metabolic acidosis with associated clinical signs of lethargy, inappetence, vomiting, muscle weakness (if hypokalemic), stunted growth, polyuria, polydipsia, and, with urolithiasis, dysuria.

In people, congenital and acquired forms are well described, acquired DRTA occurring most commonly with certain autoimmune disorders (e.g., systemic lupus erythematosus), nephrotoxicities (e.g., amphotericin B), nephrocalcinoses (e.g., primary hyperparathyroidism), and renal tubulointerstitial disorders (e.g., pyelonephritis, obstructive uropathy, interstitial nephritis, rare paraproteinemias). Mechanisms of diminished distal H^+ excretion in these disorders are somewhat speculative. Characterized congenital forms arise from mutations in genes encoding protein subunits of luminal membrane H^+-ATPase or the basolateral Na^+-HCO_3^- anion exchanger (AE1), which are necessary for appropriate H^+ excretion and HCO_3^- resorption, respectively (Figure 2). One of the described mutations in the gene for H^+-ATPase causes concurrent early onset sensorineural deafness (inner ear pumps are affected), while the other may cause late-onset deafness or no deafness. Mutations in the gene for AE1, which is expressed on erythrocyte membranes (band 3 anion transport protein), may cause DRTA or ovalocytic or spherocytic erythrocyte changes with or without hemolytic anemia. Specifically, elliptocytosis, macrocytosis, and increased erythrocyte rigidity occur in so-called southeast Asian ovalocytosis (SAO) caused by such a mutation; there is selection pressure for this disorder because it protects against erythrocyte entry by *Plasmodium falciparum* through pores formed by band 3 clusters. People with SAO and a coexistent hemoglobinopathy may develop concurrent DRTA and hemolytic anemia with ovalocytosis (Khositseth 2008).

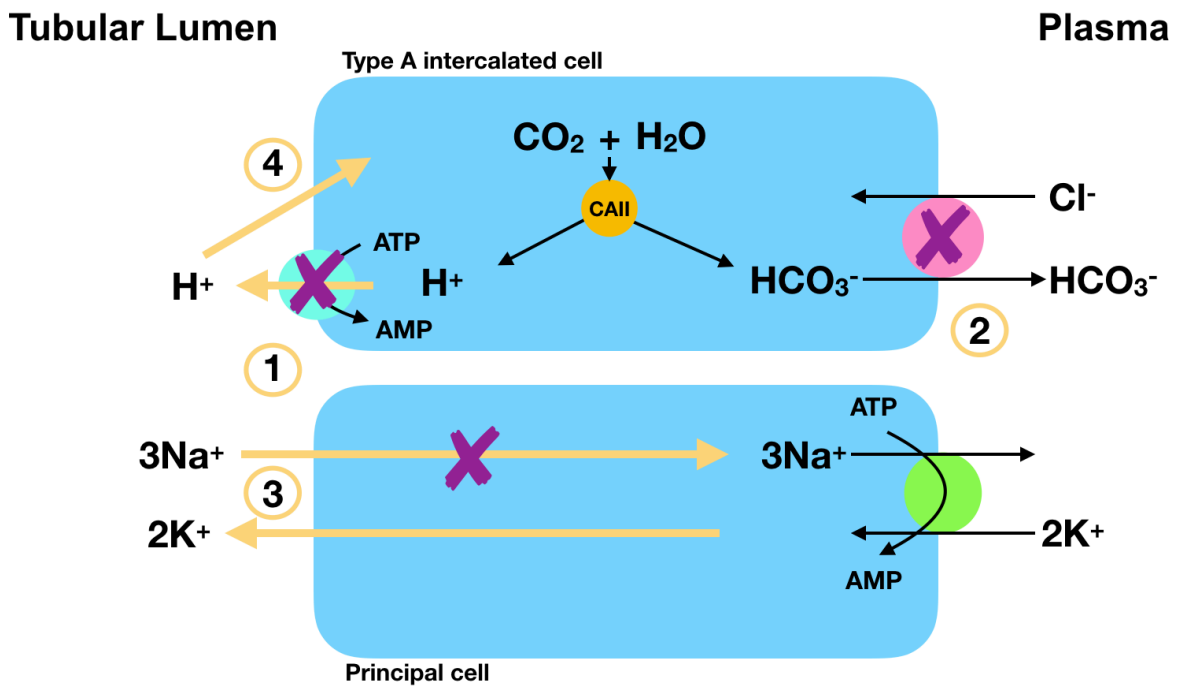

Figure 2. Major mechanisms that may contribute to DRTA

1. Decreased functional H^+-ATPase in the luminal membrane of type A intercalated cells results in decreased luminal [H^+] and therefore decreased HCO_3^- generation via carbonic anhydrase II (CAII), decreased renal excretion of Cl^-, and promotion of hyperchloremic metabolic acidosis; CAII deficiency has been associated with DRTA, but contrary to this case, it is generally associated with osteopetrosis and cerebral calcification
2. Decreased functional AE1 (HCO_3^-/Cl^- exchanger) may cause excessive intracellular accumulation of HCO_3^- with a consequent reduction in the dissociation of carbonic acid and hence reduced availability of H^+ for secretion into the tubular lumen; this impairs Cl^- excretion and promotes hyperchloremia
3. Impaired Na^+ resorption in principal cells decreases the distal luminal negative transepithelial charge potential promoting H^+ and K^+ retention and therefore metabolic acidosis and normo- to hyperkalemia
4. Damage to the tubular membrane by amphotericin B has been implicated in causing H^+ back-leak and facilitation of K^+ transfer into the lumen (K^+ wasting)

Which of these specific mechanisms contributed to the moderate to marked hyperchloremic non–anion-gap metabolic acidosis in this dog is unknown, but renal loss of HCO_3^- exceeded H^+ excretion and caused inappropriate alkalinuria in the face of acidemia.

Congenital DRTA has not been reported in dogs, but acquired forms have been associated with leptospirosis, zonisamide administration, and immune-mediate hemolytic anemia. The dog in this report may have had DRTA for years given the history of polyuria, polydipsia, and urolithiasis, so a congenital form cannot be excluded. The dog was not deaf and did not have a history of poor growth as may occur with some congenital forms of DRTA in people, but it did have unexplained abnormalities in the erythron that could conceivably have been related (see below). If the condition was acquired, an associated condition was not apparent from clinical or laboratory findings over 2.5 yr from presentation. Renal biopsies were not done.

The cause of the initial mildly regenerative, macrocytic, hypochromic anemia was thought to be mild extravascular hemolysis as there was no evidence of blood loss at that time, but no specific cause was apparent. In retrospect, a relationship to DRTA is possible as the dog could have had a congenital defect in RBC and renal tubular epithelial cell AE1, although in people, anemia is not expected unless there is also a hemoglobinopathy (Khositseth 2008). Mild unexplained ovalocytosis was present and persistent in this dog. As noted, affected people generally do not have concurrent DRTA and erythrocyte abnormalities, but it does occur. Anemia associated with concurrent SAO and a hemoglobinopathy has improved with correction of the metabolic acidosis, and except for week 26, which was shortly after a complicating urinary tract infection, anemia did appear to improve with treatment in this dog. However, an AE1 defect was not considered at the time or tested for, and such a mutation has not been reported in dogs.

The free hypercalcemia (whole blood) at presentation and 2 weeks later was evaluated further to assess for underlying hyperparathyroidism or humoral hypercalcemia of malignancy that may have led to DRTA. Evidence was not found for either. Vitamin D testing was not done, but there was no apparent endogenous or exogenous source, and the findings spanned a change in diet. Chronic metabolic acidosis has been shown to increase bone resorption, and osteomalacia has been attributed to DRTA in people (Alexander 2019; Lopez 2004). The release of phosphate from bone may be helpful in DRTA as it acts as a buffer in blood and contributes to the renal excretion of H^+. Although metabolic acidosis can increase PTH secretion, release of Ca^{2+} from bone also limits its secretion. Of note, the heparinized whole blood normalized [fCa^{2+}] stayed within reference interval, but when serum was tested by a method that included initial acidification of the sample, the pH-normalized [fCa^{2+}] was repeatedly mildly increased. Discrepant same-day samples suggest falsely decreased whole blood results, falsely increased serum results, or inappropriate reference intervals.

Chronic metabolic acidosis not only increases bone resorption, but it also promotes renal calcium excretion by impairing distal tubular Ca^{2+} resorption independent of PTH or filtered Ca^{2+} load (Alexander 2017). These factors promote calcium urolithiasis, which occurred in this dog several months before presentation to MSU. Although not documented in this dog, DRTA is associated with hypocitraturia, another risk factor for calcium-based uroliths because of increased availability of Ca^{2+} when citrate is decreased. In people with DRTA, uroliths are generally made of calcium phosphate, but calcium oxalate and struvite urolithiasis were reported in one dog with DRTA (Alexander 2019; Polzin 1985).

This dog's USG was 1.008-1.019 throughout monitoring, and the dog's owners reported incontinence that may have reflected polyuria. Incontinence reportedly improved in association with a change to a low-sodium diet, but concurrent urine specific gravity results were not available. Hypercalciuria may have impaired ADH-mediated H_2O resorption in the collecting ducts and inhibited proximal tubular sodium reabsorption, thus promoting renal water loss. Sodium diuresis disrupts the normal countercurrent mechanism that allows for urinary concentration. However, other tubulointerstitial causes of impaired renal concentrating ability may have been present.

Several other laboratory abnormalities were detected at initial presentation. There was a mild leukocytosis and neutrophilia with a mild left shift, mild monocytosis, and marked lymphopenia supportive of inflammation with or without concurrent cortisol effects. The inflammatory leukogram resolved with excision of the pyogranulomatous shoulder lesion and inflamed follicular cyst. The moderate hypoferremia may also have related to the inflammatory response. Inflammatory suppression of albumin synthesis could also have contributed to the mild hypoalbuminemia, but it was persistent throughout monitoring, even when inflammation was

resolved. There were no clinical or laboratory findings to suggest hemorrhage, protein-losing enteropathy, protein-losing nephropathy, or liver disease. Chronic metabolic acidosis has been shown to reduce albumin synthesis in people (Ballmer 1995), and this may have been a significant contributing factor. Mild hypomagnesemia and total hypoproteinemia were attributable to hypoalbuminemia. The very mild hyperamylasemia was considered insignificant.

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American Society for Veterinary Clinical Pathology
CASE DISCUSSION
CASE 3

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SPECIMEN: Laboratory data (CBC, serum biochemistry, PT/APTT).

SIGNALMENT: 3-year-old male neutered Domestic Shorthair.

HISTORY AND CLINICAL FINDINGS: The patient was presented to the emergency service at the University of Minnesota Veterinary Medical Center for evaluation of acute trauma. Immediately prior to admission, the cat was found lying on the floor and was suspected to have been attacked by one of the dogs in the house. On presentation the patient was minimally responsive, approximately 6% dehydrated, tachypneic (RR of 45 brpm) and hypothermic with a temperature of 97.6°F (34.8°C). Pulse and heart rate were within reference intervals. Bruising, multiple abrasions and skin punctures were noted as well as subcutaneous emphysema and edema in the region of the left semimembranosus muscle. A mandibular symphyseal fracture was present with suspected dislocation of the right temporomandibular joint. Neurologic exam findings included tetraparesis, conscious proprioception deficits in the front limbs, a lack of palpebral response in the right eye, and absent menace response bilaterally. The rest of the physical exam was unremarkable.
Computed tomography (CT) imaging of the skull, thorax, abdomen, and pelvis were performed. CT confirmed fracture of the mandibular symphysis with an additional fracture in the left mandibular ramus and a nondisplaced fracture of the left hamulus of the pterygoid bone. Within the thorax was patchy to alveolar increased attenuation of the caudal lung lobes attributed to pulmonary contusions. Additional CT findings included focal penetrative caudal abdominal body wall trauma with trace amounts of free intra-abdominal gas and pelvic and pelvic limb soft tissue thickening associated with either edema or cellulitis secondary to trauma.

Venous blood was submitted for CBC and serum chemistry.

LABORATORY DATA:

Table 1: CBC (ADVIA® 2120 Hematology Analyzer, Siemens) with manual differential from presentation.

TEST	UNITS	RESULT	REFERENCE INTERVAL
HCT	%	29.6	29.5-47.0
RBC	x 10^6/µL	6.54	6.44-10.36
HGB	g/dL	10.0	9.8-16.8
MCV	fL	45.3	37.4-50.4

MCH	pg	15.3	12.0-18.0
MCHC	g/dL	33.7	32.1-39.7
RDW	%	16.9	No Range
Retic #	x 10^6/µL	0.029	0.004-0.066
PLT	x 10^3/µL	Estimate: 80-100	110-413
WBC	x 10^3/µL	9.19	1.83-16.27
Neutrophil Segs	x 10^3/µL	5.70	1.2-13.2
Neutrophil Bands	x 10^3/µL	1.38 H	0.0-0.16
Lymphocytes	x 10^3/µL	1.93	0.2-9.4
Monocytes	x 10^3/µL	0.18	0.0-0.8
Eosinophils	x 10^3/µL	0.00	0.0-1.9
Basophils	x 10^3/µL	0.00	0.0-0.3

Sample Appearance: Slightly icteric
RBC Morphology: 1+ Acanthocytes, rare keratocytes, rare Howell-Jolly bodies
WBC Morphology: 3+ Toxic change, 2+ Dohle bodies, rare reactive lymphocytes
PLT Morphology: Many small clumps, appears decreased, 2+ large platelets

Table 2: Serum Chemistry data (Beckman Coulter AU480 Chemistry Analyzer) from presentation.

TEST	UNITS	RESULT	REFERENCE INTERVAL
BUN	mg/dL	66 H	12-39
Creatinine	mg/dL	2.4 H	0.5-2.1
Calcium	mg/dL	4.6 L	8.3-10.9
Phosphorus	mg/dL	12.3 H	3.3-7.8
Magnesium	mg/dL	4.8 H	1.6-2.4
Total Protein	g/dL	4.6 L	5.9-8.2
Albumin	g/dL	2.1 L	2.4-4.1
Globulin	g/dL	2.5	2.5-5.3
Sodium	mmol/L	141 L	147-158
Chloride	mmol/L	109 L	113-123
Potassium	mmol/L	4.9	3.9-5.3
Bicarbonate	mmol/L	68.3 H	12-20
Osmolality		303	298-319
Anion Gap		-31 L	19-30
Bilirubin, Total	mg/dL	2.0 H	0.0-0.3
ALP	U/L	28	2-88
GGT	U/L	<3	0-3
ALT	U/L	364 H	16-127
AST	U/L	2780 H	14-42
CK*	U/L	668559 H	54-744
Glucose	mg/dL	142	74-143
Cholesterol	mg/dL	97	56-226
Amylase	U/L	653	555-1600
LIH Indices		0 0 2	No Range

*Measurements for this analyte are considered linear up to 20,000 U/L. Concentrations above this value may not be exact.

Table 3: Coagulation Panel (ACL TOP® CTS 300 Coagulation Analyzer, Instrumentation Laboratory) from presentation.

TEST	UNITS	RESULT	REFERENCE INTERVAL
PT	Seconds	10.7 H	8.2-9.9
APTT	Seconds	34.0 H	10-21.9
Fibrinogen	Mg/dL	177	77-206

QUESTIONS:

1. What process(es) is/are causing the markedly high serum bicarbonate concentration?
2. What diagnostic test(s) could be performed to support your suspicions?

ADDITIONAL FINDINGS:

Upon evaluation of CBC and biochemistry results, additional testing was performed.

Blood gas panel with lactate (i-STAT® 1, Abbott Diagnostics)-venous sample

TEST	UNITS	RESULT	REFERENCE INTERVAL
pH		7.203	No Range
pCO_2	mmHg	46.6	No Range
pO_2	mmHg	21.0	No Range
HCO_3	mmol/L	18.3	No Range
Glucose	mg/dL	93	No Range
BUN	mg/dL	80	No Range
Creatinine	mg/dL	2.4	No Range
Sodium	mmol/L	135	No Range
Potassium	mmol/L	4.6	No Range
Chloride	mmol/L	109	No Range
Total CO_2	mmol/L	20	No Range
Anion Gap		12	No Range
Ionized Calcium	mg/dL	2.9	No Range
Lactate	mmol/L	1.73	No Range
HCT	%	27	No Range
HGB	g/dL	9.2	No Range

Pertinent urinalysis data-collection method: cystocentesis

TEST	RESULTS
Color	Light red
USG	1.029
pH	7.0
Turbidity	Clear
Glucose	Negative
Bilirubin, dipstick	1+
Bilirubin, ictotest	Positive
Ketones	Negative
Occult blood	3+
Protein, dipstick	3+
RBC	5-20/HPF
WBC	Occasional/HPF

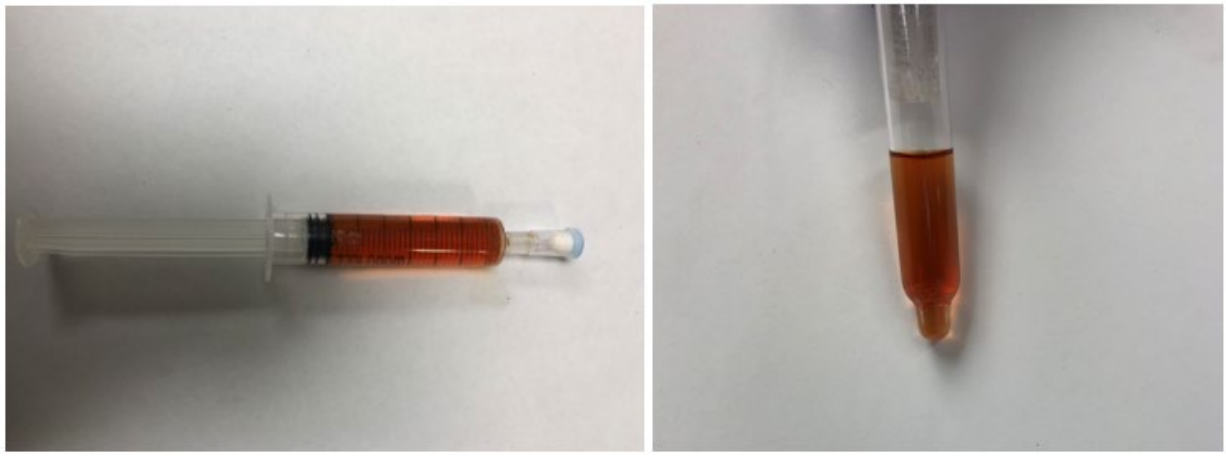

Figure 1: Gross appearance of urine obtained by cystocentesis from a 2-year-old DSH with marked muscle trauma. The image on the left shows the urine prior to centrifugation. Following centrifugation (right image) the urine remains clear and dark red.

Lactate dehydrogenase (LDH) levels were also measured and were markedly increased at 75,552 U/L. Feline reference interval: 35-325 U/L. LDH assay is linear up to 1200 U/L. Values above this level may be not be accurate.

INTERPRETATION/DIAGNOSIS:
1. Marked spurious increase in bicarbonate, negative anion gap, and electrolyte and mineral derangements associated with severe rhabdomyolysis.

2. Probable DIC

OUTCOME/FOLLOW-UP: Following triage treatment for the patient's injuries, the owners elected humane euthanasia due to extent of trauma and uncertain prognosis. No post-mortem evaluation was performed.

ANSWERS TO QUESTIONS: Answers to questions will be provided in the discussion section.

DISCUSSION: Rhabdomyolysis is a serious condition resulting from severe necrosis or breakdown of myocytes due to direct or indirect skeletal muscle trauma. The resultant release of massive quantities of intracellular contents can lead to life-threatening sequelae-most notably renal injury-and multiple abnormal clinical parameters.[1,2] The classic clinicopathologic pattern observed in patients with rhabdomyolysis generally reflects this release of cellular constituents including marked increases in serum CK and AST (with possible increases in ALT), hyperkalemia, hyperphosphatemia, and myoglobinuria.[2] Hypocalcemia is also a common finding, suspected to be the result either of rapid shifting in response to peracute increases in phosphorus or the influx of calcium into damaged cells.[2,3] In our patient, the severe muscle damage resulting from the dog attack led to expected increases in CK, AST, and ALT as well as hyperphosphatemia and hypocalcemia (both total and ionized measurements). Interestingly, a marked increase in serum bicarbonate and negative anion gap were also observed. As the latter abnormalities are not considered compatible with life, an interference reaction was strongly suspected associated with the measurement of serum bicarbonate.

The Beckman Coulter AU480 Chemistry Analyzer, along with many other chemistry analyzers, determines serum bicarbonate concentrations using a two-step enzymatic method (Figure 2). In the first step, bicarbonate and phosphoenolpyruvate (PEP) are converted to

oxaloacetate and dihydrogen phosphate in a reaction catalyzed by the enzyme phosphoenolpyruvate carboxylase (PEPC). Following this reaction, malate dehydrogenase (MD) catalyzes the reduction of oxaloacetate to malate in addition to the oxidation of nicotinamide adenine dinucleotide (NADH). The oxidation of NADH results in a decrease in absorbance at the 340nm wavelength which, when measured spectrophotometrically, is proportional to the bicarbonate concentration.[4,5,6] The presence of marked elevations in either pyruvate or lactate dehydrogenase (LDH) results in a known interference with this method. Following severe muscle damage, release of LDH and pyruvate results in an additional reaction whereby LDH catalyzes the conversion of pyruvate to lactate and the oxidation of NADH. Under normal conditions, oxamate competitively inhibits LDH by forming a ternary enzyme-NADH-oxamate complex. With massive release of LDH associated with myocellular injury however, this inhibitory action can be overcome resulting in increased consumption of NADH leading to an artificially high serum bicarbonate concentration and, in this patient, a spuriously decreased calculated anion gap.[5,6] (Answer to Question 1).

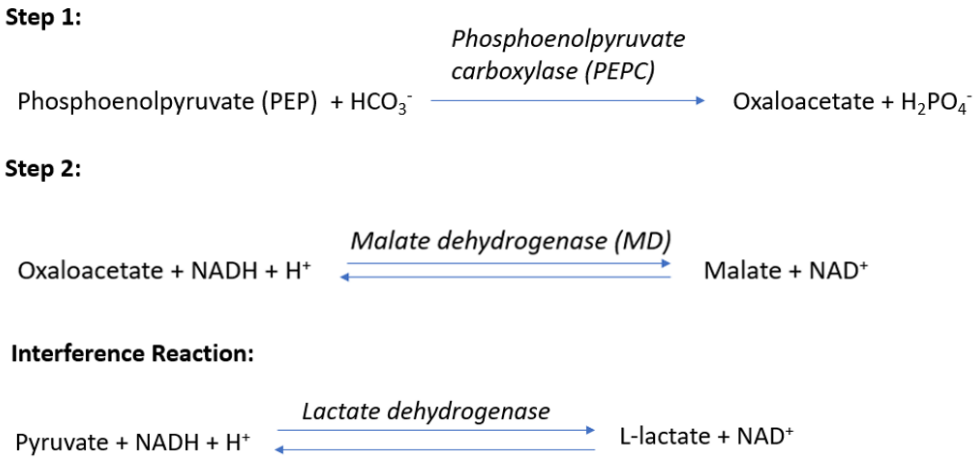

Figure 2: Enzymatic reactions involved in the determination of serum bicarbonate concentrations by spectrophotometry.

In contrast to the majority of chemistry analyzers that employ enzymatic reactions to determine bicarbonate concentration, the iSTAT and other blood gas analyzers utilize ion-specific electrodes to measure pH and pCO_2 which are used to calculate bicarbonate concentration from the Henderson-Hasselbach equation.[5] These measurements are not affected by the presence of elevated pyruvate or LDH concentrations and thus are considered to be a more accurate measurement of bicarbonate in the face of severe myocellular injury. Bicarbonate concentrations and anion gap values within reference interval on blood gas analysis, along with marked elevations of LDH in our patient, were consistent with spurious bicarbonate increases due to LDH interference as well as an erroneous AG calculation. (Answer to Question 2).

Spurious increases in serum bicarbonate secondary to rhabdomyolysis have primarily been reported in horses and cattle and may be more common in these species due to their large muscle mass.[5,6] However, rare case reports have indicated that small and exotic animal species are also affected by this phenomenon. A mystery case presented at the AVCP conference in 2015 reported suspected LDH interference resulting in discrepant bicarbonate concentrations between chemistry and blood gas analyzers and a lower than expected anion gap in a Labrador Retriever diagnosed with heat stroke.[7] To the author's knowledge however, LDH was not measured in this case to confirm the suspected interference. Lastly, a case report recently published in the *Journal*

of Avian Medicine and Surgery described erroneously high bicarbonate, negative anion gap, and markedly increased LDH concentration in an African Parrot resulting from rhabdomyolysis of undetermined etiology.[8]

Other clinicopathologic findings in this patient were thought to be directly or indirectly related to marked whole body trauma and will be discussed in further detail below.

CBC findings in this patient were consistent with inflammation (regenerative left shift with toxic change) and probable emerging DIC (thrombocytopenia, erythrocyte morphologic changes). Mild prolongation of PT and moderate prolongation of APTT added support to the concern for DIC though further hemostatic testing (FDPs, d-dimers) were not pursued. DIC is a recognized potential sequela to rhabdomyolysis in humans and is thought to result from activation of the coagulation cascade by components released from damaged myocytes.[1,8] Finally, given the borderline low hematocrit as measured by the ADVIA and the mild decreased hematocrit determined by the iSTAT, it is probable that dehydration is masking a mild anemia. The extent of tissue injury and evidence of mild hemolysis suggest that hemorrhage within damaged muscle and/or vascular injury respectively may be occurring in this patient. Furthermore, peracute hemolysis could also be a minor contributing factor in the development of DIC.

Biochemical abnormalities were consistent with marked tissue injury and dehydration. Moderate azotemia was primarily attributed to pre-renal causes given the reported 6% dehydration on physical examination. However, in light of the inappropriately concentrated urine, a renal component was also considered probable. Causes of renal azotemia considered included direct kidney trauma, ischemic injury, and myoglobinuric renal failure. The presence of dark red pigmenturia that did not resolve with centrifugation (Figure 1) was attributed to myoglobinuria. Myoglobin has nephrotoxic properties and the deposition of this protein within renal tubules can result in renal failure, although the peracute nature of the injury makes this etiology less likely.[1] Ammonium sulfate precipitation testing to confirm myoglobinuria was not performed. Hemoglobinuria could not be eliminated as contributing to the pigmenturia as hemolyzed serum was noted; however, as the Beckman Coulter chemistry analyzer grades hemolysis on a six-point scale, the 2+ hemolysis was not considered sufficient to be a major contributor to pigmenturia. Additionally, the positive heme strip and 2+ proteinuria were primarily attributed to myoglobinuria although the presence of low numbers of erythrocytes noted on the sediment and possible free hemoglobin could have contributed to both of these positive reactions. Furthermore, due to the mild hypoalbuminemia, glomerular protein loss could not be excluded. Unfortunately, necropsy was not performed to assess for renal lesions.

Mild hypoalbuminemia may have been secondary to protein-rich fluid loss and/or hemorrhage within the necrotic areas of damaged muscle as observed by the presence of subcutaneous edema in the pelvic limbs. Additionally, inflammatory mediators released from damaged myocytes could induce microvascular permeability changes resulting in extravascular loss of proteins. Finally, inflammation leading to a negative acute phase response may be playing a minor role in the hypoalbuminemia although this response generally takes several days to develop.

As mentioned above, the patient had mineral derangements (moderate hyperphosphatemia and marked hypocalcemia) that were primarily attributed to massive myocyte damage. Hypermagnesemia is a less common, though recognized finding in patients with rhabdomyolysis.[1] Additionally, decreased glomerular filtration rate (GFR) is likely contributing to the hyperphosphatemia and hypermagnesemia. Hypocalcemia due to decreases in the protein-bound fraction secondary to hypoalbuminemia was also considered to be a factor; however the ionized calcium was also decreased, indicating that this was a minor contributor to the hypocalcemia. Mild to moderate proportionate decreases in sodium and chloride are primarily related to acute myocyte injury allowing influx of these electrolytes (similar to calcium) from the extracellular fluid to the intracellular space, although third space loss or hemorrhage resulting in fluid shifting could not be ruled out. Interestingly, hyperkalemia was not observed in our patient.

Although considered an expected finding with acute muscle damage, upon review of previous case reports, hyperkalemia is not a consistent finding in rhabdomyolysis patients with several animals displaying serum potassium levels within the reference interval.[5,6,7,8]

The acid-base status of this patient was challenging to assess. The bicarbonate and total CO_2 concentrations, as determined by blood gas, were within reference interval although the pH did indicate a mild acidemia. The lack of a high anion gap (based on blood gas analysis) or hyperchloremia did not support a metabolic acidosis. A mild increase in pCO_2 suggests a respiratory acidosis, possibly secondary to pulmonary injury; however, pCO_2 levels do trend higher in venous samples compared to arterial blood (arterial blood gas was not available) therefore respiratory contributions are difficult to assess adequately. Patients with rhabdomyolysis frequently exhibit a high anion gap (titrational) metabolic acidosis usually attributed to lactic acid and potentially uremic acid accumulation.[1,2,8] Our patient's lactate was only mildly increased and may reflect the peracute presentation.

The mild hyperbilirubinemia and bilirubinuria indicate possible emerging hepatobiliary disease. The moderate increases in ALT and AST were mainly attributed to musculoskeletal injury, however a degree of hepatocellular damage due to ischemic or direct injury cannot be eliminated as a possibility leading to early cholestasis.

In summary, our case details a known interference of LDH in the spectrophotometric measurement of serum bicarbonate in a DSH cat secondary to marked skeletal muscle injury. While this interference has been reported most frequently in large animals, this case serves as a reminder that small animal species can also present with this abnormality. Our case further illustrates the importance of blood gas analysis in critically ill patients and recognition and proper interpretation of improbable values on blood chemistry analysis. Knowledge of this interference is of particular importance to emergency veterinarians and clinical pathologists charged with assisting in the interpretation of biochemical data.

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American Society for Veterinary Clinical Pathology
CASE DISCUSSION
CASE 4

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SIGNALMENT: 3 year old, female spayed, German Shorthaired Pointer

HISTORY AND CLINICAL FINDINGS:

Korra was first presented to CSU Urgent Care for acute development of multiple subcutaneous masses and a presumptive diagnosis of multiple myeloma based on marked hyperglobulinemia. A week prior Korra was taken to a local veterinary clinic for decreased appetite, vomiting, hematochezia, and gingival bleeding. Over the course of three days prior to presenting at CSU, Korra developed multiple subcutaneous masses.

At presentation Korra was quiet, alert, and responsive and her vital parameters were within normal limits. Her mucous membrane color appeared pale-to-pink with a capillary refill time of 1 second. She had a palpable large ventral thoracic subcutaneous mass (25 x 12 cm) with surrounding bruising and a large dorsal thoracic subcutaneous mass (10 x 6 cm), as well as edema in the left forelimb. The masses were suspected to be hematomas. A SNAP 4DX test was performed with a negative result and thoracic radiographs were unremarkable. An abdominal ultrasound showed diffuse splenic heterogeneity with a well-defined, round and irregularly marginated 3.5 cm in diameter splenic mass. There was also mild hepatic lymphadenomegaly and a small volume of anechoic peritoneal effusion. The remainder of the structures and organ parenchyma were within normal limits.

LABORATORY DATA:

Table 1. Hemogram results from initial complete blood count (Siemens ADVIA 120 Hematology Analyzer).

TEST	UNITS	RESULT	REFERENCE INTERVAL
Nucleated Cells	10^3/ul	19.1 H	4.5 - 15.0
Neutrophil#	10^3/ul	5.5	2.6 - 11
Lymphocyte#	10^3/ul	13.4 H	1 - 4.8
Monocyte#	10^3/ul	0.2	0.2 - 1.0
Plasma Protein	g/dL	10 H	6.0 - 7.5
HCT	%	25 L	40 - 55
RBC	10^6/ul	3.71 L	5.5 – 8.5
HGB	g/dL	8.4 L	13 - 20
HGB (cell)	g/dL	8.1 L	13 - 20

MCV	fl	66	62 - 74
RDW	%	17.5 H	12 - 15
MCHC	g/dL	34	33 - 36
Reticulocytes	10^3/ul	48.6	0 - 100
CH-Retic	mg/dL	26.9	22.3 - 27.9
MCV-Retic	fl	96	78 - 100
Platelets	10^3/ul	88 L	200 - 500
MPV	fl	14.5	7.5 – 14.6
Clumped Platelets		Few	

Table 2. Presenting serum biochemistry results (Roche Cobas c501Serum Chemistry Analyzer).

TEST	**UNITS**	**RESULT**	**REFERENCE INTERVAL**
Glucose	mG/dL	134 H	70 - 115
BUN	mG/dL	33 H	7 - 30
Creatinine	mG/dL	1.2	0.6 - 1.6
Phosphorus	mG/dL	6.3 H	2.5 - 6.0
Calcium	mG/dL	10.7	9.0 - 11.5
Magnesium	mG/dL	1.7 L	1.8 - 2.4
Total Protein	G/dl	10.1 H	5.0 - 7.0
Albumin	G/dl	1.4 L	3.0 - 4.3
Globulin	G/dl	8.7 H	1.5 - 3.2
A/G ratio		0.2 L	0.9 - 2.4
Cholesterol	mG/dL	49 L	130 - 300
T-bilirubin	mG/dL	1.2 H	0.0 - 0.2
ALP	IU/L	28	15 - 140
ALT	IU/L	17	10 - 90
AST	IU/L	11 L	15 - 45
CK	IU/L	32 L	50 - 275
GGT	IU/L	0	0 - 9
Sodium	mEQ/L	144	142 - 152
Potassium	mEQ/L	3.78 L	3.9 - 5.4
Chloride	mEQ/L	103.4 L	108 - 118
Bicarbonate (HCO3-)	mEQ/L	14.4 L	15 - 25
Anion Gap	mmol/L	30 H	12 - 23
Calculated Osmolality	mOsm/Kg	294	
Iron	uG/dL	138	80 - 270
Hemolysis	mG/dL	6	0 - 100
Icterus	mG/dL	0	0 - 1
Lipemia	mG/dL	9	0 - 80

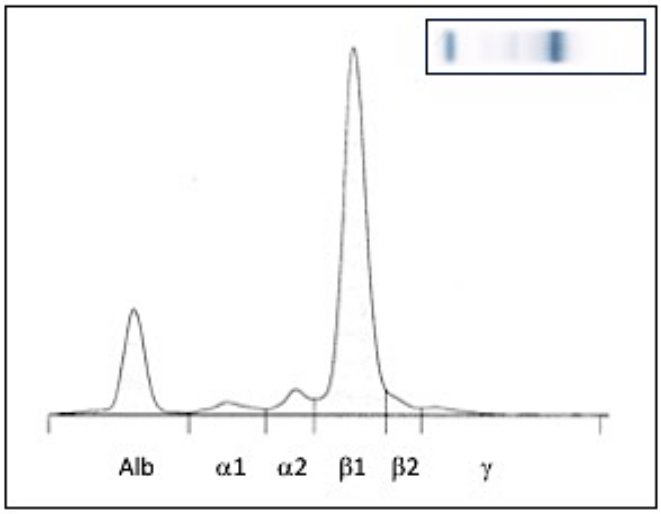
Table 3. Initial clotting test results (STA Compact Max analyzer Diagnostica Stago).

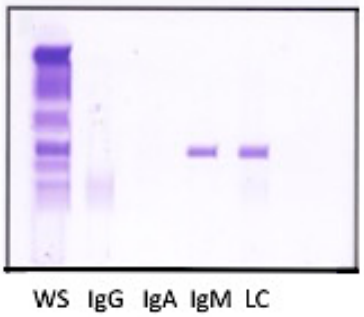
TEST	UNITS	RESULT	REFERENCE INTERVAL
Protime	Sec	12.8 H	7.4 - 9.4
APTT	Sec	19.9 H	9.8 - 13.3
FDP		NEG	
D-Dimer	ug/ml	0.12	0.03 - 0.4
AT3	%	67 L	104 - 162
Quantitative Fibrinogen	mG/dL	66 L	123 - 210

Table 4. Urinalysis on initial presentation.

TEST	RESULT
Sample method	Voided
Volume	5 mL
Color	Yellow
Clarity	Slightly hazy
Specific Gravity	1.012
pH	6
Protein	Neg
Glucose	Neg
Ketones	Neg
Bilirubin	1+
Blood	Trace
Squamous	None
Transit/Renal	None
WBC	None
RBC	None

ADDITIONAL TESTS:





A/G Ratio: **0.21** T. P.: **10.1** g/dl

Fractions	%	Conc.	Int. ref. g/dl
Albumin	**17.3**	**1.75**	**2.77 - 3.74**
Alpha 1	**4.0**	**0.40**	**0.23 - 0.38**
Alpha 2	**5.2**	**0.53**	**0.67 - 1.16**
Beta 1	**68.1**	**6.88**	**0.37 - 0.81**
Beta 2	**3.0**	**0.30**	**0.39 - 0.81**
Gamma	**2.4**	**0.24**	**0.33 - 0.76**

Figure 1. Serum protein and immunofixation electrophoresis data from a 3 year old, female spayed, German Shorthaired Pointer. Alb = albumin, WS = whole serum, LC = light chain.

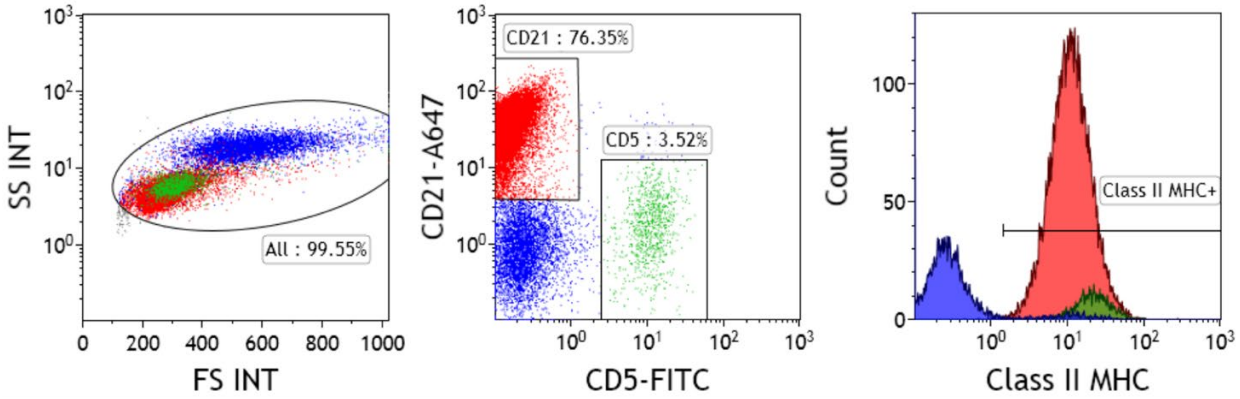


Figure 2. Peripheral blood flow cytometric findings on a 3 year old, female spayed, German Shorthaired Pointer at initial presentation. The forward scatter (FS) axis is presented on a linear scale and the side scatter (SS) axis is presented on a logarithmic scale. CD21+ cells are labeled red, CD5+ cells are labeled green. All cells are CD45+ and CD34- (data not shown).

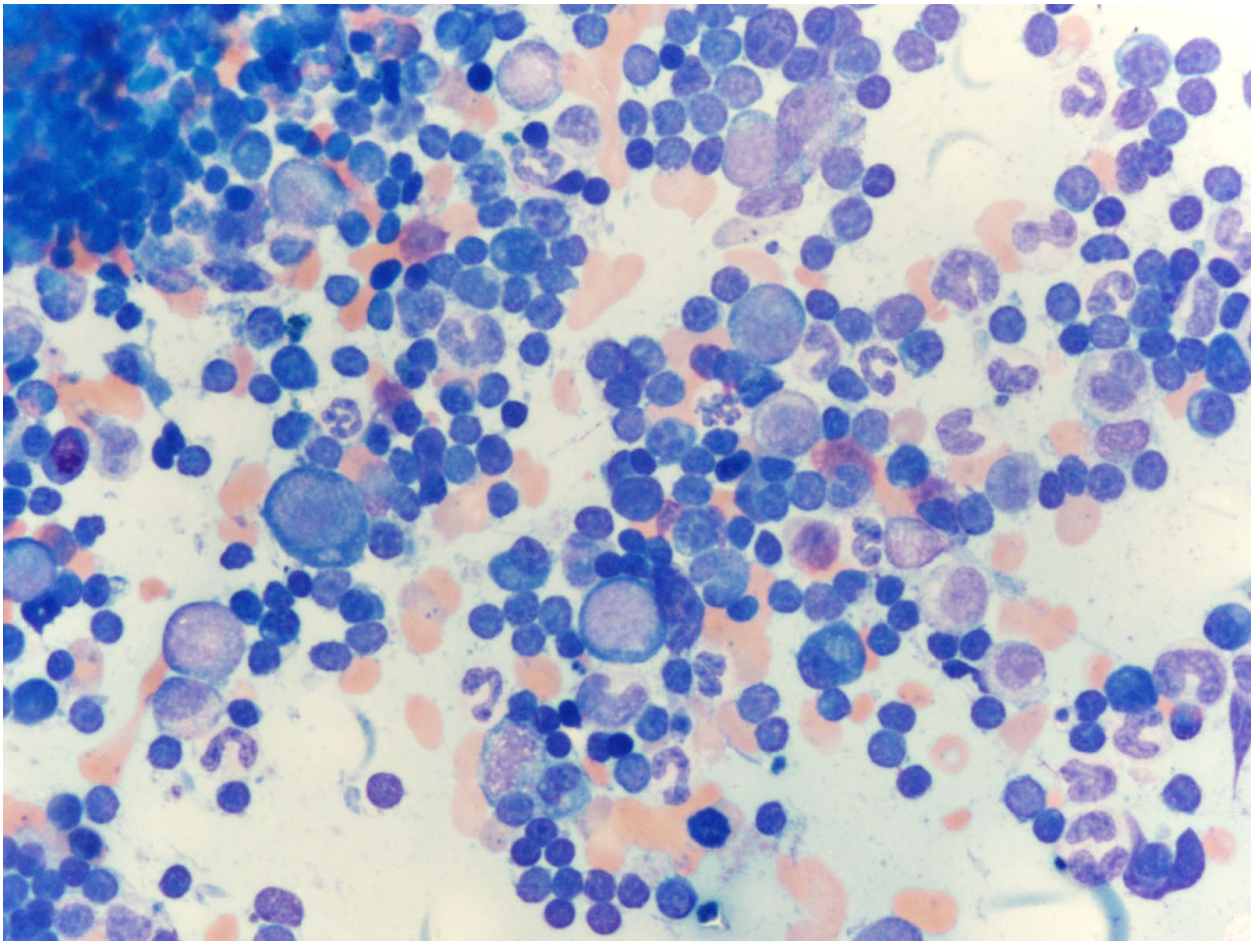


Figure 3. Bone marrow aspirate (50x objective; Wright-Giemsa) from a 3 year old, female spayed, German Shorthaired Pointer. The bone marrow particles are highly cellular against a thick basophilic background with 10-25% adipose tissue at lower magnification (not shown).

QUESTIONS:

1) Combining the clinical, flow cytometry, bone marrow and electrophoresis findings, what is the most appropriate diagnosis?
   a. Acute lymphocytic leukemia
   b. B cell CLL/Waldenstrom's macroglobulinemia
   c. Reactive lymphocytosis
   d. Multiple myeloma

2) Paraproteinemia may interfere with chemistry analyzer measurement of which of the following analytes?
   a. Potassium and albumin
   b. Albumin and T-bilirubin
   c. Potassium and T-bilirubin
   d. ALP and T-bilirubin

INTERPRETATION/DIAGNOSIS:

- B-CLL/Waldenstrom's macroglobulinemia with IgM monoclonal gammopathy
- Artifactual hyperbilirubinemia
- Artifactual hypoalbuminemia

ADDITIONAL FINDINGS:

SERUM PROTEIN AND IMMUNOFIXATION ELECTROPHORESIS DESCRIPTION
An electrophoretic gel and tracing and immunofixation gel are reviewed. There is a moderate hypoalbuminemia. There is a distinct restricted band in the β-1 globulin fraction which labels with IgM heavy chain and light chain by immunofixation. This band makes up 6.3 g/dL of protein. There is slightly decreased β-2 and γ globulin fractions, possibly representing suppression of normal immunoglobulin as there is minimal IgG heavy chain and IgA heavy chain labeling by immunofixation.

BONE MARROW CYTOLOGIC DESCRIPTION
The sample is of high cellularity on a thick blue background (consistent with hyperglobulinemia) with a large amount of blood. Bone marrow particles are hypercellular with 10-25% adipose tissue, and there are large numbers of hematopoietic cells scattered through the background. There are scant iron stores. There are increased numbers of megakaryocytes, with mild left shifting. There are 80% lymphocytes, which are predominantly small with a small round nucleus, condensed chromatin, no apparent nucleoli and a small amount of basophilic cytoplasm. There are rare intermediate sized lymphocytes with more dispersed chromatin and slightly expanded pale blue cytoplasm. In a 500 cell differential, there are 4% mature well differentiated plasma cells, though their distribution is patchy through the sample. Myeloid maturation is complete and left-shifted; there are small numbers of immature myeloid precursors (26%) and mature myeloid precursors (10%). There are very scant erythroid precursors, including one rubriblast and two metarubricytes in a 500 cell differential. There are rare macrophages which are vacuolated and contain globular dark green-black pigment consistent with iron.

FLOW CYTOMETRY DESCRIPTION
The flow cytometry study revealed a homogeneous expansion of small B cells which expressed CD21 and MHC II, consistent with a diagnosis of lymphoproliferative disease, such as B-CLL.

CONFIRMATORY BIOCHEMISTRY PROFILES (Ortho-Clinical Vitros Fusion 5,1, Purdue University College of Veterinary Medicine Clinical Pathology Laboratory)

TEST	UNITS	RESULT	REFERENCE INTERVAL
Total Bilirubin	mG/dL	0.30	0.10-0.80
Unconjugated Bilirubin	mG/dL	0.0	0.0-0.30
Conjugated Bilirubin	mG/dL	0.0	0.0-0.0
Delta Bilirubin	mG/dL	0.30	0.0-0.70
Total Protein	G/dL	10.3 H	4.8-6.9
Albumin	G/dL	3.0	2.3-3.9
Globulin	G/dl	7.3 H	1.7-3.8
A/G ratio		0.4 L	0.8-1.9

REFRACTOMETRIC TOTAL PROTEIN = 10.5 g/dL

MANUAL TOTAL BILIRUBIN ASSAY The Roche automated bilirubin assay (Bilirubin Total Gen.3, Roche Diagnostics, Indianapolis, IN) performed on the Cobas c501 was performed manually so that the reaction could be visually observed. Briefly, 1.2 ml of reagent A and 0.24 ml of reagent B were mixed with 20 µl of serum and allowed to react for 5 minutes at 37°C (see Figure 4). Four canine serum samples were evaluated. A) normal T-bilirubin sample (0.1 mg/dL, RI = 0.0-0.2 mg/dL), B) increased T-bilirubin due to hepatobiliary disease (7.9 mg/dL, RI = 0.0-0.2 mg/dL), C) Korra's serum, and D) serum from another dog with a marked IgM monoclonal gammopathy. The volumes of the reagents and serum used in tube "D" were proportionally reduced due to reagent scarcity.

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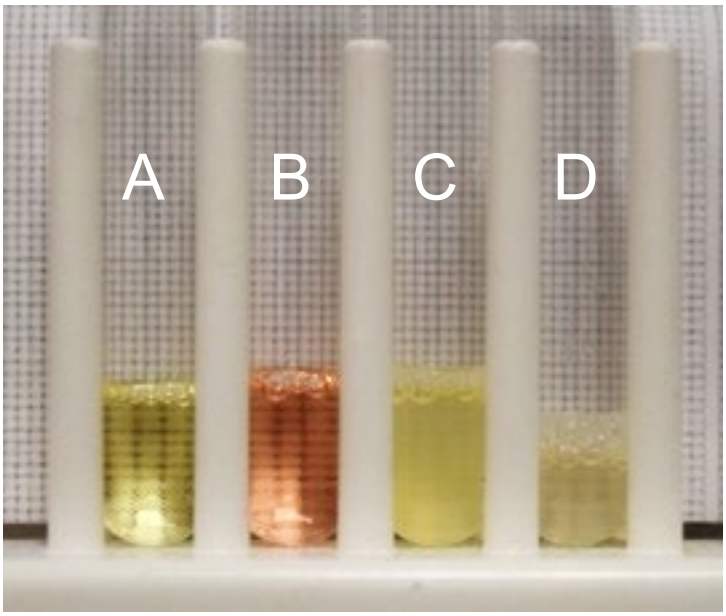

Figure 4 The Roche automated bilirubin assay was performed manually on A) normal T-bilirubin sample (0.1 mg/dL, RI = 0.0-0.2 mg/dL), B) increased T-bilirubin due to hepatobiliary disease (7.9 mg/dL, RI = 0.0-0.2 mg/dL), C) Korra's serum, and D) serum from another dog with a marked IgM monoclonal gammopathy.

OUTCOME/FOLLOW-UP: The patient was started on chlorambucil and prednisone. Clinical signs resolved and her total protein and globulin fraction decreased and were within normal limits at her 6-month check-up (6.6 g/dL and 2.9 g/dL, respectively). The restricted (M-protein) band was discernably decreased by SPE, but still measurable at 0.6 g/dL. At her 2 year check up with the CSU Oncology department, Korra was reportedly doing well at home. A CBC was performed which revealed no significant abnormalities. Her chemistry showed some elevated liver enzymes (ALP 673 IU/L, ALT 184 IU/L, AST 51 IU/L) thought to be due to chronic steroid administration. Her serum protein electrophoresis showed a stable monoclonal gammopathy. Her globulin levels have remained within the normal reference interval for the past year and T-bilirubin and albumin results have been normal. Follow-up flow cytometry was also performed on blood which showed a lymphopenia.

ANSWERS TO QUESTIONS:

1) B
2) B

See discussion for explanations of answers.

DISCUSSION:

This is a case of B-cell chronic lymphocytic leukemia (B-CLL)/Waldenstrom's macroglobulinemia (WM) which highlights the potential for paraproteins to interfere with the routine measurement of other analytes.

Classically, canine B-CLL is seen in older dogs, with a predisposition in small and toy breeds.[1] Neoplastic cells are typically small with a mature appearance and express surface CD21+ by flow cytometry. These cells commonly infiltrate the bone marrow and cause a significant peripheral lymphocytosis. Median survival time for B-CLL in dogs is ~480 days with small cell size, as defined by flow cytometry, and older age both associated with a longer survival times.[1] Similar to human B-CLL, a paraneoplastic monoclonal gammopathy has been reported in dogs. The incidence of hyperglobulinemia in canine B-CLL has been reported to range from 26-32% while one study of 22 dogs with CLL which were not immunophenotyped, 15 (68%) had a monoclonal gammopathy.[1]

The diagnostic criteria for WM in humans, also known as lymphoplasmacytic lymphoma with IgM monoclonal gammopathy, is bone marrow infiltration by small lymphocytes showing plasmacytoid or plasma cell differentiation, IgM monoclonal gammopathy, and certain cell surface markers including IgM, kappa or lambda, CD19, CD20, and weak CD22. CD5 and CD23 are usually negative. People with WM can have a higher serum IgM concentration than B-CLL and clinical bleeding is often encountered.[2] WM is poorly characterized in the dog with only few cases of WM being reported. [3]

B-CLL may mimic WM clinically in people so cellular morphology and immunophenotyping is necessary to distinguish these conditions. While lymphocytosis can occur in WM, it is reported relatively infrequently. Aberrant CD5 expression is routinely used in people to distinguish B-CLL from other B-cell lymphoproliferative disorders but CD5 positivity is not found in canine B-CLL.[4] Gene expression profiling of WM in people revealed a genotype that is more similar to CLL than multiple myeloma, suggesting that there might not be clear distinctions in certain cases.[5] The distinction between these two diseases in the dog is unclear.

The lymphocytosis characterized by the predominance of small, CD21+, MHC class II+ cells by flow cytometry, bone marrow infiltration and findings of an IgM monoclonal gammopathy in a patient with clinically evident bleeding, support a diagnosis of B-CLL or WM. This case demonstrated the small B-cell lymphocytosis classically seen with B-CLL but is atypical for canine B-CLL as the patient was a younger (3 years old) German Shorthaired Pointer. The patient's thrombocytopenia and clinical evidence of a bleeding diathesis are commonly found in human WM but peripheral lymphocytosis would be less typical. Ultimately, the patient responded well to therapy (chlorambucil and prednisone) as it is still alive more than 2 years after the initial diagnosis.

Several mechanisms have been invoked to explain the bleeding diathesis associated with paraproteinemia. In some cases of marked IgM paraproteinemia, bleeding is considered a component of hyperviscosity syndrome caused by the high molecular weight and positive charge of IgM.[2] Excessive fibrinolysis and fibrinogenolysis have been reported in several cases of multiple myeloma in humans, and the pathogenesis may be related to reduced α2-antiplasmin or complex formation of the paraprotein with plasmin.[2] Paraproteins with anti-factor

VIII or anti-thrombin activity have also been documented.[6,7] The autoantibody activity produces a clotting factor deficiency and is associated with a prolonged aPTT and bleeding diathesis. Paraproteinemia has also been shown to inhibit platelet aggregation through platelet surface coating.[8] Conversely, data suggests that some coagulation test results may be factitiously abnormal. One human case of an IgMκ paraprotein from MGUS (monoclonal gammopathy of unknown significance) exhibited antibody activity against phospholipids, bound cardiolipin and interfered with the coagulation assay in vitro but was not associated with in-vivo bleeding tendency.[9]

Laboratory clotting test abnormalities were present in this case, including hypofibrinogenemia, decreased antithrombin, and prolonged PT and PTT. These theoretically could have been artifact caused by the paraproteinemia. However, there were clinical hemostatic abnormalities such as development of hematomas and mucosal bleeding, consistent with a true bleeding diathesis. The exact mechanism of the bleeding diathesis in this presented case is unknown. Interestingly, after initiation of treatment, her IgM concentration decreased relatively rapidly, her clotting test results normalized, and she no longer had clinical evidence of bleeding.

Paraprotein interference in automated chemistry analyzers has been reported to affect multiple methods.[10,11,12] Some interference is due to the fact that paraproteins can aggregate at extremely acidic or alkaline pH or low ionic strength in an idiosyncratic manner. It is theorized that differences in amino acid sequence and relative concentration of paraprotein determines the amount of paraprotein precipitation.[13] Significant precipitation and aggregation causes turbidity which can scatter light and interfere with the photometric or absorbance measurements. [10,13,14] Paraprotein aggregates also have the potential to randomly float through the light path causing fluctuating absorbance measurements.

The increased serum T-bilirubin value is discordant with the clinical picture and other diagnostic test results. Hyperbilirubinemia secondary to hepatocellular disease was considered unlikely as there was no other evidence of hepatobiliary or cholestatic disease in the biochemistry profile and an abdominal ultrasound did not note hepatobiliary abnormalities. Pre-hepatic cholestasis was considered similarly unlikely because the CBC and blood smear show no evidence of hemolytic disease. There was a normochromic normocytic anemia without reticulocytosis or spherocytosis which is more likely due to either anemia of chronic disease, neoplasia induced myelophthisis, chronic low-grade blood loss secondary to coagulopathy or hyperviscosity induced erythrocyte destruction, or a combination of these conditions. Importantly, the patient was not clinically icteric and the serum icterus index, which typically mirrors the bilirubin value, was 0. Both human and veterinary literature suggest that paraprotein induced artifactual hyperbilirubinemia should be suspected when the sample is not visibly icteric.

Given that the Roche total bilirubin assay used to initially evaluate the patient's sample operates in a strongly acidic environment, two methods were used to confirm the suspected artifactual hyperbilirubinemia. First, the automated T-bilirubin assay was performed manually so that results could be more easily evaluated. In the Roche method a solubilizing agent, 3,5-dichlorophenyl diazonium, and bilirubin combine in the presence of a strongly acidic medium to produce a red azo dye which can be measured photometrically. When the reaction was performed manually, the expected red color was observed only in the truly hyperbilirubinemic sample while the samples from the two gammopathy patients had noted turbidity but no color change, consistent with a paraprotein induced artifactual hyperbilirubinemia.

Additionally, the same sample of the patient's serum was evaluated using a dry chemistry method at Purdue University. In dry chemistry methods, protein aggregates can still form but the layered construction of the dry slide separates protein aggregates away from the indicator layer, where reflectance is measured and results are determined.[14] The total bilirubin measured by dry chemistry methods was within the reference interval further supporting an

artifactual hyperbilirubinemia due to paraprotein interference. In health, a slightly higher bromocresol green (BCG) albumin than SPE determined albumin is expected. This occurs because BCG binds both albumin and globulin. Normally albumin reacts rapidly with BCG and peak color development occurs in less than 5 seconds but BCG binds globulins slower. The automated assay read time is kept short to minimize the amount of albumin over-estimation due to globulin binding. Paraproteins can induce a discordance between BCG albumin and SPE albumin by several methods. A factitious BCG albumin decrease can occur when the paraprotein delays albumin binding to BCG. In one human report, the serum IgM paraprotein was found to significantly delay the binding of BCG to albumin with peak color development taking several minutes.[15] A factitious SPE albumin increase can also occur when paraproteins remain in solution during the total protein assay but are not accounted for during electrophoresis. Cryoglobulin associated precipitation after total protein measurement or other sample handling abnormalities which affect loading of paraprotein in the electrophoretic gel can selectively decrease the proportion of paraprotein in the sample. Alternatively, excess protein loading in the gel can produce a band concentration which is outside the linearity range of the dye used for densitometric measurement. In all of these situations, the globulin percentage is factitiously low, the albumin percentage is factitiously high and, when albumin percentage is multiplied by an accurate total protein, the SPE albumin is factitiously high.

In this case, the expected relationship of BCG and SPE albumin is not observed. SPE albumin concentration is higher (1.75 g/dL) than BCG albumin (1.4 g/dL). Sample handling was appropriate to prevent interference from cryoglobulinemia and precipitation was not noted during loading of the gel. The sample was diluted prior to electrophoresis to ensure that the protein band was within the linearity range of the dye. Interestingly, the dry chemistry albumin was nearly 2 times the BCG albumin determined on the Cobas and was notably higher than the SPE albumin. A factitious decrease of biuret total protein, and therefore falsely decreased SPE albumin is possible because the biuret method for determining total proteins is performed at very alkaline conditions (pH ~ 12-13) which can lead to similar precipitation problems as observed with the total bilirubin reaction. Falsely low total protein can be ruled out as the wet chemistry, dry chemistry and refractometric total protein are within acceptable agreement. The mechanism for the discrepancy between the wet chemistry and dry chemistry albumin measurements are unknown but this discrepancy may suggest over-estimation of the albumin by the dry chemistry method. Ultimately, the BCG and SPE albumin discord is interpreted to be a falsely low BCG albumin.

In summary, this case represents B-CLL/WM with a monoclonal gammopathy diagnosed in an unusually young dog with a bleeding diathesis and thrombocytopenia. It is also important to recognize the possible analytical interferences that paraproteinemia, such as this patient's IgM monoclonal gammopathy, can create. In particular, false T-bilirubin and albumin values from certain chemistry analyzers as well as the possibility for various effects on the assessment of hemostasis can occur. Lastly, this case provides a good review of different methods of protein measurement and highlights the differences between dry and wet chemistry analyzers.

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