Lexie – An All Americanum Girl

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History and Presentation:

Lexie is an approximately 4-year-old female spayed, medium sized (17 kg), mixed breed dog who was transferred to the MSU-CVM Internal Medicine Department on August 28, 2020 for further work-up of bloody diarrhea, generalized muscle atrophy, lethargy, inappetence, and abnormal bloodwork, including a marked leukocytosis. During the month prior to presentation, Lexie's owner noticed loss of muscle mass, with the muscles of her head most notably atrophied. She also had a decreased appetite and had occasional bouts of anorexia for days at a time. Diarrhea with melena was appreciated throughout this month as well. Lexie also had a known history of hunting and ingesting small mammals, and did not consistently receive flea and tick preventative.

Lexie initially presented to her primary veterinarian at an unknown date for diarrhea and weight loss. At this time, her condition was thought to be due to a significant worm burden. She was prescribed an unknown probiotic and given an unknown dewormer, with no improvement in gastrointestinal signs following treatment. On Thursday, August 27th, Lexie became notably weaker and began falling into her food and water dishes and laying in her own urine. She was taken to Emergi-Pet in Northport, Alabama. Bloodwork at this visit showed a non-regenerative anemia, marked leukocytosis, hypocalcemia, hypoglycemia, and hypoproteinemia. A SNAP cPLI was within normal limits and no ova were seen on fecal flotation. At this time, Lexie was transferred to the Animal Health Center at Mississippi State for further work-up.

On presentation to MSU-CVM, Lexie was quiet, alert, and responsive. She weighed 17 kg with a body condition score of 3 out of 9. Her vitals were as follows: heart rate of 124 beats per minute, respiratory rate of 28 breaths per minute, and a temperature of 100.4°F. Her mucous membranes were pink and tacky with a capillary refill time of 2 seconds. A prominent skin tent was appreciated and she was estimated to be 8% dehydrated. A small amount of yellow

mucopurulent ocular discharge was noted bilaterally. On cardiothoracic auscultation, no murmurs, arrhythmias, crackles, or wheezes were appreciated. Significant sarcopenia was appreciated, most notably of the temporal and masseter muscles and hindlimb muscles, with easily palpable spinous processes and pelvic bones. Lexie appeared ataxic and walked very tentatively, as if she were walking on eggshells. The ataxia was attributed to the significant muscle loss at this time as no neurologic deficits were appreciated. However, a full neurologic exam was not performed. There was no obvious peripheral lymphadenopathy aside from subjectively enlarged popliteal lymph nodes bilaterally. Lexie was stabilized with intravenous fluids to account for dehydration and losses, dextrose supplementation for hypoglycemia, and Dexamethasone SP to address the differential of a potential Addisonian crisis. Following stabilization, Lexie was transferred to the Internal Medicine Department on the morning of August 28th, 2020.

Diagnostic Approach and Considerations:

Upon presentation to the Internal Medicine Department on August 28th, the top differentials for Lexie's ailments included Addison's disease, liver dysfunction, round cell neoplasia, protein-losing enteropathy (intestinal parasites, *Giardia*), and hepatozoonosis. An ammonia tolerance test was performed and ruled out hepatic dysfunction. An ACTH stimulation test subsequently ruled out Addison's disease.

The complete blood count and serum chemistry on presentation most notably revealed a marked hypoglycemia, moderate anemia that appeared poorly regenerative, mild hypocalcemia, and marked panhypoproteinemia. An ionized calcium was within reference interval; thus, the calcium result on the serum chemistry was attributed to the hypoproteinemia. Lexie's blood urea nitrogen (BUN) and creatinine were mildly to moderately decreased; these were tentatively

ascribed to decreased protein intake secondary to anorexia and decreased muscle mass, respectively. The sample of blood was submitted for pathologist review with the blood smear revealing a marked inflammatory leukogram characterized by a mature neutrophilia (86,553 /ul), increasing the index of suspicion for *Hepatozoon*. A poorly regenerative anemia was seen with no evidence of blood parasites or immune-mediated hemolytic anemia, and was likely due to anemia of inflammatory/chronic disease. A direct fecal smear and fecal flotation were performed with no ova or parasites appreciated at this time.

The Radiology reports for abdominal and thoracic radiographs revealed mild smooth periosteal proliferation of the proximal ribs, femoral diaphysis, humeral diaphysis, and thoracic spinous processes. An abdominal ultrasound was performed as well and revealed a mildly diffusely hypoechoic liver, a heterogenous echogenicity of the spleen with numerous smoothly marginated, hypoechoic nodules, and scant free fluid in the abdomen. Numerous intraabdominal lymph nodes measured mild to moderately enlarged and hypoechoic. These findings supported our differentials of round cell neoplasia (as with lymphoma or mast cell tumors) and hepatozoonosis (infection/inflammatory etiology).

As an effort to differentiate between our now top two differentials, an ultrasound-guided fine needle aspirate of the spleen and liver was performed. Cytology of the spleen illustrated minimal evidence of extramedullary hematopoiesis and significant amounts of blood contamination. Cytology of the liver revealed a mixed population of inflammatory cells (neutrophils, macrophages, and lymphocytes), with the neutrophils most significantly increased. There was significant blood contamination of the sample. Mild hepatic lipidosis was appreciated; however, there were no infectious agents, evidence of neoplasia, or inflammation present at the time of review. Additionally, lymph node aspirates were obtained and were compatible with lymphoid

hyperplasia with plasmacytosis secondary to antigenic stimulation, with either infectious or non-infectious agents considered possible.

After considering Lexie's history of eating and hunting small mammals, inconsistent flea and tick prevention, and diagnostic findings, we elected to presumptively treat Lexie for American canine hepatozoonosis. To confirm this presumptive diagnosis, a sample for diagnosis of hepatozoonosis using polymerase chain reaction (PCR) was submitted to an outside lab. On September 2nd, 2020, the PCR results came back as a high positive for *Hepatozoon americanum.*

Pathophysiology:

Hepatozoonosis is a tick-borne infection caused by an apicomplexan parasite, a type of protozoa that may result in subclinical, acute, or chronic disease in affected dogs. *Hepatozoon canis* is the main cause of canine hepatozoonosis, which results in mild disease of the hemic and lymphatic system in canids in Africa, Asia, southern Europe, and South America: more recently, this organism has also been reported in the United States. *Hepatozoon americanum*, on the other hand, is the most prevalent species affecting dogs in the southeastern United States, and is the primary cause of American canine hepatozoonosis (ACH).[1] Since Lexie was diagnosed with ACH, this will be the species of focus in this case discussion.

Hepatozoon americanum undergoes an indirect life cycle. The definitive host is *Amblyomma maculatum*, otherwise known as the Gulf Coast tick. Transmission occurs when canids, the intermediate host, ingest the adult or nymph tick vector with infective sporozoites or a paratenic host, such as rodents and rabbits, with cystozoites. The sporozoites penetrate the intestine moving into circulation, and subsequently enter the skeletal and cardiac muscles. Here, they form an "onion skin cyst" where they undergo merogony, which is asexual reproduction. The muscle cyst ruptures and releases the mature merozoites resulting in a severe inflammatory response.

Merozoites enter leukocytes where gametogony occurs. The Gulf Coast tick ingests the leukocytes with gamonts during a blood meal. Sporogony, sexual reproduction, follows within the tick completing this complex life cycle.[2, 3]

In the literature and clinically, *Hepatozoon americanum* appears to cause more severe clinical findings than *H. canis*.[3] Patients with ACH often present febrile and lethargic. Myalgia and hyperesthesia over the paraspinal regions, gait abnormalities, and muscle atrophy most markedly of the temporal muscles are also commonly seen.[4] Mucopurulent ocular discharge is often seen when the patient is febrile. Clinical signs may be seen acutely or have a waxing and waning chronic pattern thought to be due to rupture of tissue cysts and release of merozoites resulting in an inflammatory response.

Common findings on blood work include a mild to marked leukocytosis consisting of a mature neutrophilia, elevated alkaline phosphatase (ALP) activity, anemia, and hypoalbuminemia. The elevated ALP is attributed to the periosteal proliferation and inflammation. The anemia is typically non-regenerative (normocytic normochromic) and secondary to chronic inflammation. The hypoalbuminemia may be caused by decreased protein intake due to anorexia, chronic inflammation since it is a negative acute phase protein, or even a protein-losing nephropathy (PLN) secondary to glomerulonephritis or renal amyloidosis. Serum blood urea nitrogen (BUN) may be decreased, unless renal disease is present which may result in an azotemia. A mild hypoglycemia (40-60 mg/dL) may be noted due to *in vitro* metabolism of glucose due to the marked increase in numbers of white blood cells in the blood sample. Although hypoglycemia, hypoalbuminemia, and low BUN mimic liver disease, bile acid assays are usually normal to mildly elevated, and ammonia tolerance assays are typically within normal

limits, ruling liver disease out. Interestingly, creatine phosphokinase (CPK) levels are typically within the reference interval despite the severe myositis secondary to disease.[3, 5, 6]

Diagnosis of ACH may be presumptive or definitive. Clinicians may presumptively diagnose a patient based on history, clinical signs, profound neutrophilia, and periosteal bone lesions, and elect to treat empirically.[1] Bone abnormalities on radiographs may show disseminated, symmetric, periosteal bone proliferation often involving the diaphyses of the proximal long bones of the limbs without destruction of the cortical bone. The cause of these lesions is unknown, but one of many hypotheses suggests that it may be secondary to stimulation by humoral factors rather than local factors.[6] Other diagnostic tests and findings may include: electromyography revealing changes suggestive of generalized polymyopathy, lymph node aspirates showing reactive hyperplasia, and bone marrow aspirates illustrating a high myeloid:erythroid ratio.[6] Identification of gamonts in leukocytes, obtaining muscle biopsies, or submitting blood samples for PCR or serology are required for definitive diagnosis. Identifying gamonts in blood smears with Romanowsky-type stain is rarely successful, with a low diagnostic sensitivity.[1] Biopsies of skeletal muscles often illustrate numerous merogonous stages (onion tissue cysts) or pyogranulomas and can be identified with hematoxylin-eosin stains or immunohistochemical procedures. Although quite invasive, muscle biopsy is the most sensitive diagnostic option for ACH, and thus is sometimes pursued. Since *Hepatozoon* gamonts circulate in the bloodstream, PCR is thought to be a useful and non-invasive diagnostic test; however, little data exists to support its sensitivity and specificity. In the case of ambiguous results, a combination of PCR and muscle biopsy can aid in making a diagnosis.[1, 6]

Treatment and Management:

Currently, there is no labeled treatment or cure for *Hepatozoon americanum*. Treatment protocols in cases of ACH are utilized to treat acute clinical signs, prolong lifespan, and prevent relapse of clinical disease. Pain control is important in the management of these patients, and nonsteroidal anti-inflammatories have shown to be effective. Parasiticides are given in conjunction to help control the infection, but unfortunately do not appear to clear the *H. americanum* meront stages within the muscle tissue (onion skin cyst).[7, 9] A combination of medications has shown the most favorable outcome when treating clinical ACH. This combination drug therapy includes trimethoprim-sulfadiazine (TMS), clindamycin, and pyrimethamine (TCP protocol) daily for 2 weeks. Following this initial therapy, oral decoquinate should be given consistently long-term (for a minimum of 2 years) to aid in preventing relapse of clinical disease.[2, 7] Other medications that have been discussed in the literature include imidocarb diproprionate and ponazuril.[8] The goal of prevention and control is reducing the risk of exposure by using tick preventatives, preventing oral ingestion of ticks while "scavenging" or grooming, and reducing predatory behaviors towards intermediate hosts.[2]

Case Outcome and Prognosis:

Lexie remained in the Intensive Care Unit (ICU) for 7 days prior to discharge. She required fluid resuscitation and a dextrose constant rate infusion until she remained normoglycemic, following numerous episodes of severe, watery diarrhea and anorexia. She improved with supportive care for the first 2-3 days, but relapsed into another hypotensive and hypoglycemic episode. This episode was treated as discussed above. At this time, Lexie was dewormed with fenbendazole, started on metronidazole, ondansetron, and Rebalance suspension. We elected to treat Lexie empirically for ACH while awaiting PCR results. Pain management was also initiated

with the administration of gabapentin and Tylenol 4 (acetaminophen with codeine) as Lexie appeared painful upon palpation of the lumbosacral spine and hindlimbs and had difficulty walking. She was started on trimethoprim-sulfadiazine (TMS), clindamycin, and pyrimethamine (TCP combination therapy) and was instructed to continue this regimen for 2 weeks. Following that 2-week period, the owner was instructed to start Lexie on oral decoquinate (Deccox) and continue long term to prevent relapse of clinical disease. They were instructed to return in 1 week for a recheck, but Lexie was unfortunately lost to follow-up.

In cases of ACH, chronic infection is common, and most patients succumb to the disease within 1 to 2 years without supportive therapy.[6] However, unlike in the past, prognosis for dogs with ACH is no longer considered guarded to poor. The use of TCP followed by decoquinate has fortunately greatly improved prognosis for affected dogs, with less severe and frequent relapses in disease.[9]

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