Canine Ehrlichiosis

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Class of 2018

Clinicopathological Conference
Presented on February 16, 2018

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Introduction

Ehrlichiosis is a tick-borne rickettsial disease with worldwide distribution including the United States, Europe, India, and Africa[5]. There are multiple species such as *Ehrlichia ewingii* and *Ehrlichia chaffeensis*, but *Ehrlichia canis* is the major causative organism of canine monocytic ehrlichiosis, and was the first species found to infect canids in Algeria in 1935[5,18]. *Ehrlichia* is a gram negative, obligate, intracellular parasite that infects monocytes, granulocytes, and platelets depending on the species causing infection, hence the name canine monocytic ehrlichiosis for *Ehrlichia canis* and canine granulocytic ehrlichiosis for *Ehrlichia ewingii*[8,18]. Disease is transmitted through a bite from an infected tick of the genus and species *Rhipicephalus sanguineus*, the brown dog tick. Zoonotic potential does exist with human tick exposure, but the dog acting as a reservoir is of little concern[20]. Due to *Ehrlichia canis* being far more clinically important and having been most studied, this case report will focus on its diagnosis and treatment.

History and Presentation

Lucy Mills, a 17-year-old female spayed Border Collie, presented to the MSU-CVM Small Animal Internal Medicine/Oncology Service on August 21, 2017 after referral from her primary veterinarian for a two-week history of bilateral mandibular lymphadenopathy. The owner reported she felt a swelling under Lucy's neck while petting her. She was taken to her primary veterinarian where the swelling was diagnosed as enlarged mandibular lymph nodes. A fine needle aspirate was taken of the lymph nodes and sent to Antech labs for review. Clindamycin was prescribed pending results of the aspirate. Reactive lymphoid hyperplasia was diagnosed by cytological evaluation, but lymphoma could not be ruled out. On recheck examination one-week later, Lucy was found to be unresponsive to therapy with clindamycin,

and the size of the lymph node swelling had increased since her initial visit; therefore, she was referred to MSU-CVM for further diagnostics.

Upon presentation, Lucy was bright, alert, and responsive. She was of adequate weight at 22.7 kilograms, having an ideal body condition score of 5/9. Her vital parameters were within normal limits. Physical examination revealed firm, moderate bilaterally enlarged mandibular and mild bilaterally enlarged prescapular and popliteal lymph nodes. Multiple masses were noted including a soft mass on the lateral to ventral chest, a mass of her left first mammary gland, and cyst-like masses present on the epidermis in the left flank region. Further evaluation revealed a grade IV/VI left, apical, systolic heart murmur, a leftward head tilt with pain on manipulation of the neck to the right, moderate nuclear sclerosis, and moderate to severe periodontal disease.

Differential Diagnosis

Diagnosis of ehrlichiosis can be challenging, especially in an asymptomatic dog with the sole complaint of enlarged mandibular lymph nodes. Due to finding multiple, bilaterally enlarged lymph nodes on physical examination, our primary differential at initial presentation was lymphoma. Differential diagnoses for dogs presenting with lymphadenopathy include lymphoma and other neoplasia such as malignant histiocytosis, tick-borne disease such as ehrlichiosis, leptospirosis, hepatozoonosis, and fungal disease such as blastomycosis or cryptococcosis. It is important to rule out these other diseases prior to establishing a diagnosis of lymphoma, and prior to starting treatment with chemotherapeutics, as these drugs are not benign. In Lucy's case, further diagnostics were required to make a definitive diagnosis and to understand the best prognosis and treatment options available.

Pathophysiology

Ehrlichia canis, causing canine monocytic ehrlichiosis, is transmitted by the bite of an infected brown dog tick, *Rhipicephalus sanguineous*, but hematological transmission can also occur through blood transfusions[3]. Warmer seasons increase the risk of infection for ehrlichiosis due to heightened tick activity, and it infects all breeds, with the German Shepherd being the most susceptible and suffering higher morbidity and mortality rates[5]. Once bitten by the tick, there is an eight to twenty day incubation period which is followed by one of three phases and a variety of clinical signs including, but not limited to, fever, lymphadenopathy, and hemorrhage[8,17]. Phases of infection include acute, subclinical, and chronic, each lasting a certain amount of time, but the differences between phases are not easily understood[11]. Monocytes tend to be the target cells of infection[18].

Canines acutely infected with ehrlichiosis typically recover with no to minimal clinical signs and mild hematological abnormalities such as thrombocytopenia and leukocytosis[4,8,15,22]. Thrombocytopenia has been attributed to four mechanisms including platelet consumption, immune-mediated destruction, sequestration in the spleen, and decreased production by the bone marrow[3]. Bleeding is the clinical hallmark of acute disease due to one or a combination of the stated mechanisms. Immunocompetency is important, because some canines may clear infection either during the acute or subclinical phase, while others may go on to develop the chronic phase. Bone marrow aplasia, bi- or pancytopenia, and high mortality caused by septicemia or severe blood dyscrasia characterize the chronic phase of disease[7,15].

Diagnostic Approach/Considerations

Early diagnosis of ehrlichiosis is important to avoid fatality caused by the chronic phase, and can be accomplished by a collection of travel history or appropriate geographical location,

clinical presentation, and laboratory investigations including hematology, cytology, serology, and polymerase chain reaction (PCR)[15]. Only a presumptive diagnosis may be made from history and clinical signs alone due to an asymptomatic or non-specific clinical manifestation of disease; therefore, laboratory diagnostics are required. A complete blood count and serum biochemistry are initially indicated. The most common abnormality (80%) seen on the complete blood count is a thrombocytopenia, followed by a non-regenerative anemia. Other abnormalities seen include neutropenia, leukopenia, and lymphopenia or lymphocytosis[7]. Serum biochemical abnormalities tend to be unpredictable, but may include hyperglobulinemia, hypoalbuminemia, and mild elevations of alanine aminotransferase and alanine phosphatase[15,17]. Cytological demonstration of *E. canis* morulae in monocytes using Romanowsky-based stain from a buffy coat smear can lead to a definitive diagnosis[14]. However, this method is insensitive in the subclinical and chronic phases due to a low parasitemia, and it lacks specificity due to irrelevant material that may be mistaken for *Ehrlichia* morulae[15]. Serology continues to be the mainstay for confirmation of exposure, with the immunofluorescent antibody (IFA) being the 'gold standard', but enzyme linked immunosorbent assays (ELISA) are also used[11]. It takes approximately seven to thirty-five days for antibodies to develop, and an IgG titer equal to or greater than 1:80 indicates exposure, but not necessarily current infection. The best way to interpret a recent infection with serology is to obtain paired serum samples and demonstrate a four-fold increase in IgG two to three weeks apart[15].

Polymerase chain reaction (PCR) may overcome diagnostic limitations that are encountered with both cytology and serology, as it is highly sensitive for confirming infection of disease as early as four to ten days post-inoculation[21]. There are several tissues that may be used for the amplification process including whole blood, bone marrow, liver, and lymph nodes[15].

Due to PCR's ability to confirm an active phase of infection, it is very reliable at determining prognosis and choosing an effective route of therapy. PCR must be performed before starting antibiotic therapy, otherwise false negative results are common.

Treatment and Management

Once a definitive diagnosis of ehrlichiosis has been made via PCR, the decision to treat is straightforward. If a definitive diagnosis has not been made via PCR, but the patient is seropositive, it can be challenging to decide whether treatment should be initiated or not. Some believe a seropositive dog exhibiting clinical symptoms of ehrlichiosis should receive treatment[18].

Doxycycline has been and is the drug of choice, recommended at a dose of 5 mg/kg orally twice a day for twenty-eight days[2,16]. Reports state that it is very effective at resolving both clinical and hematological abnormalities, but that it is unable to eliminate *E. canis*. One study demonstrated that dogs treated with doxycycline to a PCR negative status that were re-exposed to ticks harboring *E. canis* became PCR positive again[13]. It has also been shown to be ineffective during the chronic phase of infection when the dog is suffering from aplastic pancytopenia, septicemia, and severe bleeding[1,15]. Limited evidence exists justifying the use of other tetracyclines.

Chronic phase cases not only require doxycycline as treatment, but typically also require supportive care. Supportive care includes fluid therapy with balanced crystalloids, blood transfusions using either packed red blood cells or whole blood, and bactericidal antibiotics[15].

There is one drug currently receiving attention as a possible alternative to doxycycline, rifampicin (rifampin). An in-vitro study showed rifampicin to be as effective as doxycycline and,

when given to two dogs with subclinical infection at a dose of 15 mg/kg orally twice a day for seven days, hematological abnormalities resolved as well as achievement of a PCR negative status[19]. Rifampicin may be a promising alternative to the historically-used doxycycline, but further studies are required to determine its safety[9].

Once treatment is complete, it is best to seek PCR status to determine treatment success, failure, or re-infection. Clinical recovery takes approximately twenty-four to forty-eight hours, and normalization of hematological abnormalities can take anywhere from one to three weeks in acutely infected canines[11]. Even though the patient may normalize early in treatment, treatment should not be terminated because elimination of *E. canis* may have not yet occurred. The most reliable method to determine clearance is to apply PCR to blood, bone marrow, and splenic aspirates four to eight weeks after treatment completion[10].

Tick control either by careful removal or the use of appropriate acaricides is the single most important measure to prevent infection with *E. canis*[10]. Tick control products shown to be effective include those containing phenylpyrazoles, pyrethroids, amitraz, and isoxazolines; however, it should be made clear that no product can completely prevent infection[12]. It has also been suggested to prophylactically use low doses of doxycycline daily in endemic areas where tick control is difficult, but this practice may eventually lead to drug resistance[6].

Case Outcome

In Lucy's case, blood and urine were both obtained for a minimum database consisting of a serum biochemistry profile, complete blood count, and urinalysis; all findings were insignificant. Fine needle aspirates were taken of each enlarged mandibular, prescapular, and popliteal lymph node for cytologic evaluation. Most samples were non-diagnostic, but

lymphoma was suspected on some samples due to an increased number of intermediate to large size immature lymphocytes and lymphoblasts. Fine needle aspirates were taken from the mass noted in the left first mammary gland, revealing peripheral blood constituents and fat, indicative of a lipoma. A neurological consultation and examination was sought and revealed pain of the neck on manipulation to the right, mildly delayed conscious proprioceptive deficits of the left hind limb, and a mild decrease in cutaneous trunci reflex. Diagnostics to determine a neurologic deficit were not currently indicated.

Lucy returned the subsequent day for imaging consisting of thoracic and abdominal radiographs, abdominal ultrasound, and an echocardiogram. Thoracic radiographs and echocardiogram revealed mild left atrial and ventricular enlargement, and moderate to severe mitral and mild tricuspid regurgitation. Chronic degenerative valve disease was diagnosed. Abdominal radiographs suggested an enlarged spleen and irregularity of the left kidney margin probably consistent with chronic infarcts. Ultrasound confirmed radiographic findings, and aspirates of both the liver and spleen were obtained for cytology. Liver aspirates were normal having no cytological abnormalities, while the splenic aspirates indicated extramedullary hematopoiesis and possible lymphoma, again suggested by an increased number of lymphoblasts. Lymph node biopsy was recommended to help confirm or rule out a neoplastic process.

The following day Lucy returned and blood was obtained for submission of an MDR1 (multi-drug-resistance-1) gene test. This test is used to confirm whether a dog has the gene mutation and if it will develop adverse reactions to certain drugs, particularly chemotherapeutics used to treat lymphoma. Lucy was MDR1 negative. Flow cytometry was performed on the mandibular lymph node aspirate and results were inconclusive, revealing a heterogenous

lymphocyte population (60% B lymphocytes and 40% T lymphocytes) and making a diagnosis of lymphoma less probable. The diagnosis was rethought and a 4DX SNAP test was submitted to reveal that Lucy was indeed antibody positive for *Ehrlichia canis/ewingii*.

Lucy returned one final day for submission of a tick-borne disease PCR panel. The owner also elected to go ahead with the lymph node biopsy rather than waiting on the PCR panel results. Lucy was placed under general anesthesia and her left mandibular lymph node was removed and submitted for histopathology. Recovery was smooth and Lucy was discharged later that evening with a two week course of doxycycline. The lymph node pathology results revealed marked lymphoid hyperplasia, and not lymphoma, indicated by a mixed population of B cells with plasma cell proliferation surrounding smaller clusters of T cells. Approximately one week later, the tick-borne PCR panel documented Lucy to be positive for *E. ewingii* DNA; therefore, a definitive diagnosis of ehrlichiosis was made based on PCR and the collection of other diagnostic findings.

Once treatment with doxycycline was initiated, the owners reported Lucy to be feeling better and acting as her normal self. She was even wanting to play with her housemate, Emily. Before completing the two-week course of doxycycline, a refill for another two weeks was prescribed by Lucy's primary veterinarian. Lucy has been doing well at home since completion of therapy.

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