Eclipse's Bloody Problem

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Introduction

Mycoplasma haemofelis previously known as *Haemobartonella felis* is a bloodborne bacterial pathogen found in the erythrocytes of cats. This bacterium is usually seen as bacteria cocci or rods within or around the edges of red blood cells, thus, may be diagnosed using a blood smear. *Mycoplasma haemofelis* is thought to be one of the most pathogenic out of other hemoplasmas seen in cats such as *Candidatus* Mycoplasma haemominutum, and *Candidatus* Mycoplasma turicensis. [(1)] *Mycoplasma haemofelis* can cause severe life-threatening anemia in cats due to the host's immune response destroying red blood cells. It is one cause of feline infectious anemia. Clinical signs in cats can include pale gums, lethargy, weakness, icterus, tachycardia, and pyrexia. Most clinical signs observed can be traced back to anemia due to a hemolytic process. Although severe signs can be seen with cats that have *Mycoplasma haemofelis,* chronic carriers are common, and many times show no symptoms at all. [(2)] The cause and transmission of *Mycoplasma haemofelis* is still not well understood. *Mycoplasma haemofelis* has been reported in both clinically healthy and ill cats in North America, Europe, Japan, and Australia. Natural infections are more likely seen in male, outdoor, mixed-breed cats. [(1)]

Signalment and History

Eclipse is an approximately 5-year-old male neutered domestic shorthair that presented to Mississippi State University College of Veterinary Medicine emergency services on the afternoon of March 14th, 2021. Eclipse has access to both indoors and outdoors and lives with 2 other cats who are both strictly indoors. He was fully vaccinated at presentation including his FELV vaccination and feline Bordetella. Eclipse presented with a history of lethargy, and inappetence of approximately five days duration. Eclipse was seen by his referring veterinarian

earlier in the week and received 300mls of subcutaneous fluids, an injection of vitamin K as well as an injection of Cerenia for nausea and vomiting. At Eclipses referring veterinarian, thoracic radiographs were performed which were unremarkable as well as an ELISA SNAP test for FELV and FIV which were both below detectable limits or negative. Eclipse had noticeable icterus and was suspected to be anemic, so his referring veterinarian recommended referral to Mississippi State University College of Veterinary Medicine emergency services immediately.

On presentation to Mississippi State University College of Veterinary Medicine emergency services, Eclipse was alert and responsive but very depressed. He weighted 4.5 kilograms with a body condition score of 5/9. He had a heart rate of 200 beats per minute with strong synchronous pulses, a respiratory rate of 88 breaths per minute and a low temperature of 98.7 degrees Fahrenheit. Upon cardiopulmonary auscultation, no crackles, wheezes, murmurs, or arrythmias were heard. His mucous membranes were dry and very icteric with a capillary refill time of over 3 seconds. He had a noticeable skin tint and was approximately 8% dehydrated. His eyes and nose were clear and free of discharge, but his skin was diffusely icteric. His abdomen was soft and non-painful on palpation. All palpable lymph nodes were small and symmetrical. His blood pressure readings were as followed: 157/97 (117), 187/66 (106), 192/105 (134). An abdominal and thoracic FAST scan was performed and showed no visible free fluid.

Diagnostic Approach

Diagnosing *Mycoplasma haemofelis* is not always as easy as it seems. Many cats are acutely infected or are carriers and yet no bacteria are seen within erythrocytes on a blood smear. *Mycoplasma haemofelis* is currently uncultivatable in vitro and does not grow on media. One study that performed genome sequencing on two hemoplasmas species also talked about glucose being the only energy source for this pathogen which is a possible cause for it being

uncultivatable. [5] Blood smear cytology is known to be unreliable for diagnosis of *Mycoplasma haemofelis*. Blood smears have a low sensitivity but a high specificity. One study reported a sensitivity of 0-37.5% but a specificity of 84-98%. [3] False positives and false negatives are also common findings with stain precipitation being the cause of false positives along with Howell-jolly bodies and artifacts. [3] The bacterial cocci seen in erythrocytes are usually on the periphery of red blood cells and thus can easily fall off and go into the extracellular matrix if the blood is left in the test tube for a prolonged period and is not analyzed fast enough. This may lead to false negatives.

Polymerase chain reaction (PCR) has the best sensitivity and specificity when diagnosing *Mycoplasma haemofelis* and is the gold standard. [4] A real-time PCR assay has also been studied and proven to detect and quantify *Mycoplasma haemofelis* DNA in blood samples from naturally and experimentally infected cats. [6] This test is performed in specialized labs after DNA extraction from submitted blood samples. The quantitative real-time PCR assay can aid in monitoring response to treatment as well, but the blood sample must be taken before any antibiotic treatment is initiated because organism numbers can fall very quickly once treatment has been implemented. [3] The bacterial numbers can fluctuate in some cats diagnosed with *Mycoplasma haemofelis* for several months after infection. This fluctuation may be due to cyclic antigenic variation that is used to hide from the host's immune system. [7]

Common findings seen in cats diagnosed with *Mycoplasma haemofelis* include regenerative anemia with polychromasia, macrocytosis and aggregate reticulocytosis, a positive Coombs test, hyperbilirubinemia, increased ALT and/or ALP, leukopenia, lymphopenia, eosinopenia, monocytosis, and pyrexia. Nonregenerative anemia may be found in cats with concurrent infections such as FeLV or FIV. [1]

Following Eclipse's initial physical exam, blood was collected and submitted for a small animal CBC, a neuro chemistry panel, a reticulocyte count, and a coagulation profile. Blood typing was unable to be performed due to autoagglutination. Eclipses CBC revealed a marked macrocytic hyperchromic anemia with a PCV of 9% (30.0-46.4%), MCV at 62.4 Fl (40.0-54.0 Fl), MCHC at 20.0pg (13.0-19.0 pg), as well as a marked hemoglobinemia at 2.6g/dl (9.1-15.2 g/dl). His RBC morphology showed 1+ Polychromasia, Slight Rouleaux, Slight / Rare Agglutination, 1+ Anisocytosis, and 1+ Polychromasia. The sample condition was 2+ icteric. Due to the severe anemia, infectious etiologies such as Mycoplasma or immune mediated hemolytic anemia were top differentials, pending a pathological review. Eclipses neuro chemistry panel revealed a moderate increase in ALT at 230 U/L (7-60 U/L), a marked hyperbilirubinemia at 8.8 mg/dl (0.1-0.5), and a moderate albuminemia at 1.8 g/dl (2.2-3.2 g/dl). Eclipse's reticulocyte count confirmed a regenerative anemia with a reticulocyte percentage of 4.1 (0.0-0.5%) and an absolute reticulocyte count of 53300 ul (30000-60000 ul). Eclipses coagulation profile was within normal limits. On pathological review the following day of presentation, *Mycoplasma haemofelis* infection was confirmed. Eclipse also had pulmonary and thoracic radiographs taken as well as an abdominal ultrasound. On ultrasound, the spleen was mildly enlarged with rounded margins and the parenchyma was diffusely mottled with occasional sharply marginated, round, homogeneously hypoechoic nodules that measured up to 1 mm in diameter. The changes to his spleen were likely due to extramedullary hematopoiesis. Splenomegaly was reported on abdominal radiographs which was also likely due to extramedullary hematopoiesis. The next day, a comprehensive anemia real-time PCR panel was sent out to confirm *Mycoplasma haemofelis* as well as evaluate for other concurrent infections including Cytauxzoon felis, Bartonella spp., Anaplasma spp., Erlichia spp.,

Candidatus Mycoplasma turicensis, FeLV, and FIV. *Mycoplasma haemofelis* was confirmed on PCR but all other infections were negative.

Pathophysiology

As stated before, the transmission process of *Mycoplasma haemofelis* is still unknown. Some theories include cat to cat transmission through biting, *Ctenocephalides felis* transmission, transmission through ticks or mosquito vectors, blood transfusions and clinically ill queens to kittens in utero, during parturition, or through nursing. [(1)] In one study, it was proven that *Ctenocephalides felis* was able to transmit *Mycoplasma haemofelis* but PCR results were only positive in one cat, and was shown as a transient infection with no clinical signs. [(8)] DNA for *Mycoplasma haemofelis* has been found in ticks infecting cats but has not yet been proven to be a mode of transmission. [(9)] It is recommended that all blood donors be PCR tested for many bloodborne infections including *Mycoplasma haemofelis* due to the potential of blood transfusion transmission. [(10)]

Although severe clinical signs can be seen in immunocompetent cats with an acute infection of *Mycoplasma haemofelis* it is not uncommon to have chronic carriers as well. *Mycoplasma haemofelis* is found to be a cyclic bacterium that has different methods of avoiding detection from the host's immune system and establishing chronic infection this is thought to be due to its antigenically dynamic cell surface which gives the ability to change its surface features. It is theorized that phase and/or antigenic variation could also be the reason for such cyclic nature and persistent infection of *Mycoplasma haemofelis.* [(11)]

Zoonotic risk of *Mycoplasma haemofelis* is also a big concern, especially in immunocompromised humans. One study describes a case report of a Mycoplasma haemofelis-

like infection in an HIV-positive patient from Brazil who also had a co-infection with Bartonella henselae. These positive results were discovered by PCR. Clinical signs seen in this patient were similar to ones seen in feline infections including anemia, loss of appetite, muscle pain, and lymph node enlargement. Theories of transmission from cat to human include cat bites and scratches as well as flea infestations of *Ctenocephalides felis*. Diseases associated with mycoplasma infections are occurring more frequently and are being seen in both immunocompromised and immunocompetent patients. Obviously, living near cats poses a greater risk of possible infection. [(12)]

Treatment

β- lactams are not an effective treatment against *Mycoplasma haemofelis* due to its lack of cell wall. The treatment of choice is Tetracycline (doxycycline), and a fluoroquinolone (marbofloxacin or pradofloxacin). The dose of doxycycline used is typically 10 mg/kg by mouth every 24 hours or 5 mg/kg by mouth every 12 hours and is usually given for 2-4 weeks or longer. [(3)] One study showed that using pradofloxacin for two weeks at 5-10 mg/kg by mouth every 24 hours was more effective than doxycycline. This regimen may also be more effective at clearing the infection. [(13)] Caution should always be taken when giving oral doxycycline to cats due to the possibility of esophageal stricture. Other supportive care such as fluids, blood transfusions, medications to increase appetite or even placing a feeding tube are important and often required in severe infections.

Albeit more difficult, it may also be possible to clear the carrier state of *Mycoplasma haemofelis.* Clearing the carrier state would be beneficial in situations of multicat households with some cats not infected with *Mycoplasma haemofelis,* blood donors, cats that are immunocompromised or housemates that are immunocompromised, and cats with owners who

are immunocompromised. One study evaluating clearing the carrier state using doxycycline orally at 5mg/kg twice daily for 28 days. Afterwards, cats that were still positive using real-time PCR testing were then given marbofloxacin orally at 2mg/kg once daily. After use of this regimen, all cats were PCR negative with 5 cats only needing doxycycline and 10 additional cats needing both doxycycline and marbofloxacin. [14]

Due to Eclipse's severe anemia, 45 mLs of Type A blood was given slowly over several hours under supervision. An accurate blood typing was unable to be performed due to autoagglutination. Veraflox (pradofloxacin) was started the same day of arrival, given orally once a day at 7.5mg/kg. The following morning, Eclipse was transferred to the Small Animal Internal Medicine department. Veraflox was continued but Cerenia at 1mg/kg, Prednisolone 20mg tablets at 2mg/kg, Mirtazapine and a LRS constant rate infusion of 17mls/hr was added to his treatment plan. Cerenia was added for vomiting, Mirtazapine for an appetite stimulant and Prednisolone to help aid in the decrease of hemolyzing red blood cells. His anemia was monitored with PCV checks at least twice daily.

Case Outcome

Over the next several days Eclipse continued to improve with his treatments. After his initial blood transfusion his PCV increased from 9% to 19%. His PCV fluctuated between 20-28% for the next several days. A second chemistry panel was performed in hospital on May 17th, 2021 which revealed improving liver enzymes and an ALT value of 150 U/L vs a value of 230 U/L on presentation. Although at discharge, his PCV dropped to 20% due to probable hemolysis of transfusion red blood cells, Eclipse was eating and doing very well in hospital and was discharged on May 18th, 2021. He was instructed to stay on oral Veraflox as directed for 3-4 weeks as well as his Prednisolone 20mg ½ tablet given by mouth every 24 hours for 1 additional

week. His owner was instructed to have a repeat PCV/TP on Eclipse performed in the next 3 days at his referring veterinarian as well as a repeat chemistry panel in the future to monitor his ALT values. At the time of diagnosis his PCR test results were still pending but were confirmed in the next several days following discharge. Eclipse has not been seen for a follow-up appointment with MSU CVM since his discharge.

References

1. W.B. Saunders. (2008). Chapter 115/Rickettsial Infections. In Handbook of Small Animal Practice (fifth edition). essay.
2. Barker EN. Update on Feline Hemoplasmosis. Vet Clin North Am Small Anim Pract. 2019 Jul;49(4):733-743. doi: 10.1016/j.cvsm.2019.02.009. Epub 2019 Apr 5. PMID: 30961999.
3. Séverine Tasker et al. and published in the Journal of Feline Medicine and Surgery 20, 2018, 256-261, http://journals.sagepub.com/doi/pdf/10.1177/1098612X18758594
4. Braddock, J. A., et al. "The Use of Real-Time PCR in the Diagnosis and Monitoring of Mycoplasma Haemofelis Copy Number in a Naturally Infected Cat." Journal of Feline Medicine and Surgery, vol. 6, no. 3, June 2004, pp. 161–165, doi:10.1016/j.jfms.2003.12.004.
5. Barker, Emily N et al. "Molecular characterization of the uncultivatable hemotropic bacterium Mycoplasma haemofelis." Veterinary research vol. 42,1 83. 12 Jul. 2011, doi:10.1186/1297-9716-42-83
6. Tasker, S., Helps, C.R., Day, M.J., Gruffydd-Jones, T.J., Harbour, D.A., 2003b. Use of Real-Time PCR to detect and quantify Mycoplasma haemofelis and 'Candidatus Mycoplasma haemominutum' DNA. Journal of Clinical Microbiology 41, 439–441.
7. Tasker, Séverine et al. "Distribution of Mycoplasma haemofelis in blood and tissues following experimental infection." Microbial pathogenesis vol. 47,6 (2009): 334-40. doi:10.1016/j.micpath.2009.09.009
8. Woods JE, Brewer MM, Hawley JR, Wisnewski N, Lappin MR (2005): Evaluation of experimental transmission of 'Candidatus Mycoplasma haemominutum' and Mycoplasma

haemofelis by Ctenocephalides felis to cats. American Journal of Veterinary Research 66(6), 1008-1012.

9. Duplan F, Davies S, Filler S, Abdullah S, Keyte S, Newbury H, Helps CR, Wall R, Tasker S (2018): Anaplasma phagocytophilum, Bartonella spp., haemoplasma species and Hepatozoon spp. in ticks infesting cats: a large-scale survey. Parasit Vectors 11(1), 201.
10. Pennisi MG, Hartmann K, Addie DD, Lutz H, Gruffydd-Jones T, Boucraut-Baralon C, Egberink H, Frymus T, Horzinek MC, Hosie MJ, Lloret A, Marsilio F, Radford AD, Thiry E, Truyen U, Mostl K (2015): Blood Transfusion in Cats ABCD guidelines for minimising risks of infectious iatrogenic complications. Journal of Feline Medicine and Surgery 17(7), 588-593.
11. Santos, A.P., Guimaraes, A.M., do Nascimento, N.C. et al. Genome of Mycoplasma haemofelis, unraveling its strategies for survival and persistence. Vet Res 42, 102 (2011). https://doi.org/10.1186/1297-9716-42-102
12. dos Santos AP, dos Santos RP, Biondo AW, Dora JM, Goldani LZ, de Oliveira ST, de Sá Guimarães AM, Timenetsky J, de Morais HA, González FH, Messick JB. Hemoplasma infection in HIV-positive patient, Brazil. Emerg Infect Dis. 2008 Dec;14(12):1922-4. doi: 10.3201/eid1412.080964. PMID: 19046522; PMCID: PMC2634649.
13. Dowers KL, Tasker S, Radecki SV, Lappin MR (2009): Use of pradofloxacin to treat experimentally induced Mycoplasma hemofelis infection in cats. Amercian Journal of Veterinary Research 70(1), 105-111.
14. Novacco M, Sugiarto S, Willi B, Baumann J, Spiri AM, Oestmann A, Riond B, Boretti FS, Naegeli H, Hofmann-Lehmann R. Consecutive antibiotic treatment with doxycycline

and marbofloxacin clears bacteremia in Mycoplasma haemofelis-infected cats. Vet Microbiol. 2018 Apr;217:112-120. doi: 10.1016/j.vetmic.2018.03.006. Epub 2018 Mar 10. PMID: 29615243.