Hemotropic Mycoplasmosis in a Cat

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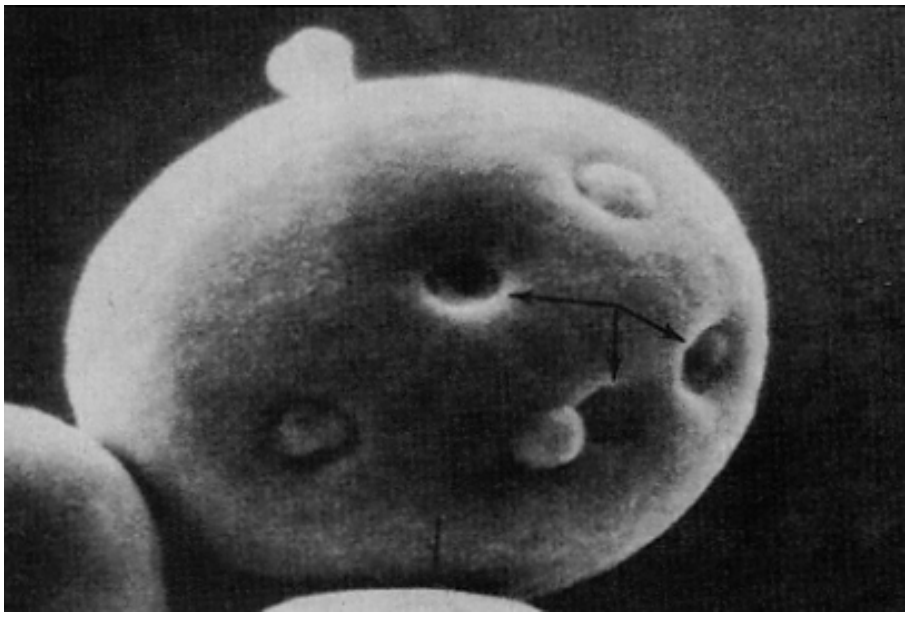


Figure 1. Electron photomicrograph of a red blood cell infected with Mycoplasma haemofelis.

Key Words: Feline Hemotropic Mycoplasmosis, Feline Infectious Anemia, Feline Haemobartonellosis, *Mycoplasma felis*, anemia

Abstract

This is a case study of Feline Hemotropic Mycoplasmosis (previously known as Feline Haemobartonellosis) in a young adult male feline with the sudden onset of signs associated with hemolytic anemia. Feline Hemotropic Mycoplasmosis (or FHM) is a "parasitic" infection of the red blood cells that results in both intravascular and extravascular hemolysis. There are two etiologic agents that infect feline red blood cells: *Mycoplasma haemofelis* (large form) and *Mycoplasma haemominutum* (small form). *Mycoplasma haemofelis* was previously known as the Rickettsial agent *Haemobartonella felis*. It was recently reclassified due to new information from PCR, Western Blot, and DNA Sequencing tests. Presenting complaint is commonly the sudden onset of behavior changes, lethargy, weakness, and/or pale, icteric mucous membranes. Diagnosis is based on blood smear evaluation. PCR assays are also available. Treatment involves a 3 week course of oral tetracyclines; doxycycline is the drug of choice at 5mg/kg once daily. Glucocorticoids may be administered to reduce extravascular hemolysis. Prognosis of uncomplicated FHM with treatment is excellent although affected cats remain carriers of the organism for life. Concurrent immunosuppression can result in death during the acute phase of the disease or recrudescence of the disease from the carrier state.

Introduction

Feline Hemotropic Mycoplasmosis (or FHM) is a "parasitic" infection of the red blood cells that causes hemolytic anemia and the associated clinical signs. There are two etiologic

agents of FHM that infect feline red blood cells: *Mycoplasma haemofelis* (large form) and *Mycoplasma haemominutum* (small form). The large form is believed to be more pathogenic while the small form is believed to be more prevalent. *Mycoplasma haemofelis* was previously classified as the Rickettsial (protozoan) agent *Haemobartonella felis* due to its inability to be cultured (not even on cat blood agar or *Mycoplasma* media), small size, and predisposition to attack red blood cells. It was recently reclassified due to new information from PCR, Western Blot, and DNA Sequencing tests, revealing its significant sequence similarity to *Mycoplasma* species. Since the responsible agent is bacterial, the condition is more accurately termed a bacteremia than a parasitemia.[1,2]

Prevalence: While reliable identification of these agents has been limited by poor specificity and sensitivity of tests in the past, it is believed that hemotropic mycoplasmas are worldwide in distribution and endemic in many areas of the United States.[1,2] Currently, any young adult male cat presenting in North America with clinical signs of anemia should be suspected of FHM.[1]

Transmission: Transmission is through infected blood. Presumably, this involves blood-feeding arthropods like ticks, lice, fleas, and mosquitoes. Cat bites are a very likely source of transmission as a large number of patients have healing bite wounds or scars.[1] Transmission can also occur via blood transfusions. Congenital infections have been documented, although it is unknown whether transmission occurred in utero, during parturition, or through the milk.[1,2,3] Although transmission by this route sounds easy, FHM positive cats in multiple cat households have not been documented to transmit the disease to their negative housemates.[2]

Risk Factors: The most likely cats to contract the organism are young adult male intact cats, mostly due to behavior that increases their exposure. These tend to be outdoor only, or indoor/outdoor cats that present in the spring and summer months. These are months when male cats roam, arthropod vectors are prevalent, and when male cats tend to fight. In addition, cats with FeLV are more likely to test positive for FHM.[a] Cats with the above signalment that present with anemia should be suspected of FHM, especially if they have history of anemia with or without clinical signs (Several factors affect the degree of parasitemia of the host and, subsequently, the severity of the anemia experienced in FHM. Many cats are believed to have been infected but never showed clinical signs).[1,2]

Pathogenesis: After inoculation with infected blood, the organism reproduces unchecked by binary fission in the vascular space during the preparasitemic phase of the disease. This phase usually lasts for 2 to 3 weeks. When the organisms attach to the red cells via adhesive fibrils they cause distortion of the red cell membrane which subsequently envelopes the organisms into pockets (Figure 1, page 1). Some infected red cells undergo intravascular hemolysis, destroyed within the vascular space by heavy parasite burden or by passing through the microvasculature with increased cell membrane fragility. This is only a small proportion of the parasitized red cells.[4]

[a] An association between Feline Hemotropic Mycolplasmosis (FHM) and FeLV was first recognized in the '70s when several studies noted a high incidence of FeLV among cats with FHM (up to 48%). In addition, cats with FeLV exhibited more extreme clinical signs of FHM including more severe parasitemia, lower PCV, and higher mortality. And vice versa: Co-infected cats developed more severe erythroid hypoplasia/aplasia than cats infected with FeLV alone. Finally, synergism also occurs between the small form of FHM (*Mycoplasma haemominutum*) and FeLV, causing clinical signs on par with clinical signs of the large form.

The primary method of red cell destruction is immune-mediated. The Host makes antibodies, primarily IgM, but also IgG, against exposed or altered red cell antigens, "tagging" infected red cells. Extravascular hemolysis then occurs in the reticuloendothelial system in the spleen, bone marrow, and liver. Macrophages of the reticuloendothelial system phagocytize infected red cells and produce the breakdown products.[4]

Intra- and extravascular hemolysis lead to clinical signs during the acute phase of FHM. The parasite burden and reticuloendothelial system (or RES) destroy a large percentage of circulating red blood cells, leading to the clinical signs of anemia as seen in Pip. The spleen plays such a large role in extravascular hemolysis that, if the parasite burden is large, it becomes engorged, resulting in splenomegaly. At the same time, it releases so much unconjugated bilirubin that hepatocyte transporters become overwhelmed, allowing unconjugated bilirubin to continue to circulate. This leads to icterus. Further description of clinical signs and associated diagnostic findings are provided in the case report.[5]

Case Report

Signalment: "Pip" was a 1.5 year old male castrated domestic shorthair feline.

Chief Complaint: "Pip" presented to the Triage Service at Cornell University Hospital for Animals on July 23, 2003 with the chief complaint of the sudden onset of hiding, lethargy, weakness, pale and icteric mucous membranes, weak femoral pulses, tachypnea, and tachycardia.

Case History: Pip had been adopted from Cortland County SPCA in June, 2003. Presumably, he was a stray prior to his arrival at the shelter. It was unknown if Pip had been outside of New York State prior to adoption, but had not left the state since his time at the shelter. Pip had undergone a standard vaccination and deworming protocol without complication. He had no history of illness since adoption other than ear mites which were successfully treated with ivermectin (0.3 mg/kg SQ 7/7/03 & 7/21/03) and milbemycin (small amount AU 7/21/03).

Clinical Findings: On initial physical examination, Pip was bright, alert, and responsive with good body condition. He had a normal body temperature and mildly elevated heart and respiratory rates. His mucous membranes were pale and mildly icteric, but moist with a capillary refill time of under two seconds. He had weak femoral pulses that were synchronous with his heart beat and a mild Grade I/VI systolic heart murmur.

Differential Diagnoses: There are three classes of differentials for anemia. These are blood loss, red blood cell destruction, and reduced red blood cell production. Anemia can also be classified based on the degree of bone marrow response, as regenerative or non-regenerative (Table 1). Pip's clinical signs, including icterus, were sudden in onset. Therefore, a hemolytic anemia was suspected.

Differential Diagnoses for Anemia

Regenerative Anemia (Acute):

- Blood Loss
  - Trauma
  - Coagulation disorder
  - GI (parasites – hookworms, ulcers, neoplasia, IBD)
  - UT (hemorrhagic cystitis, neoplasia, renal)
- RBC destruction (Icterus)
  - IMHA
  - Blood parasites (**hemotropic mycoplasmas, cytauxzoon, babesia**)
  - Abnormal RBCs (lead poisoning, hereditary)
  - Chemical/toxin (Heinz body anemia, snake venom)

Non-regenerative Anemia (Chronic):

- Reduced RBC production
  - Nutritional (iron/B12/folate deficiency)
  - Chronic disease (renal, Addison's)
  - Aplastic anemia (toxin, **ehrlichia**, bone marrow neoplasia/disease)
  - Pure RBC aplasia (FeLV, immune, drug, idiopathic)
  - Congenital

Table 1. Classification of Differential Diagnoses for Anemia (adapted from Merck Veterinary Manual Online, *www.merckveterinarymanual.com/mvm/index.jsp*; 2003)

Diagnostic Tests (only significant abnormal findings are reported): QATs revealed that Pip was severely anemic (PCV 16%) and his serum was icteric, indicating hemolysis. His blood did not agglutinate as can be seen in FHM, but this did not rule out the disease. He was found to have Type A blood. Blood smear evaluation (Diff-Quik) showed a strongly regenerative anemia (mild anisocytosis, few basophilic stippled RBCs, mild polychromasia), indicated a possible bacterial infection (mild toxic changes in neutrophils), and exhibited moderate *Mycoplasma haemofelis* organism (0.3um - 0.8um diameter epierythrocytic coccoid, rod-like, or ring-shaped organisms, some in short chains) (Figure 2).[1,2]

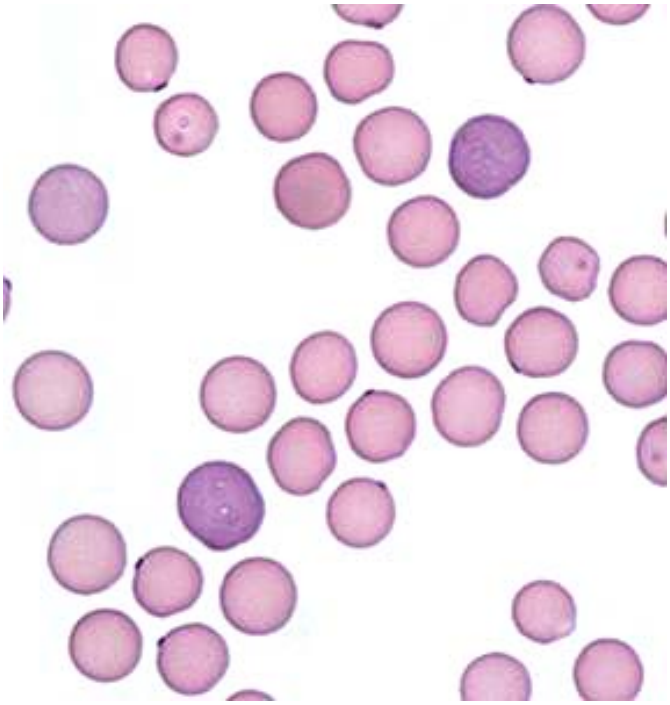


Figure 2. Blood Smear showing a regenerative anemia and *Mycoplasma haemofelis*, as described in the text.[3]

Complete blood count confirmed a regenerative anemia [PCV 15% (32-52), Retics 3.2% (0-0.1%), Retics (abs) 92.8 thou/uL (8.6-55.8), Nucl RBCs 12/100 WBC (0/100)], and suggested a bacteremia [Bands 0.8 thou/uL (0-0.1)], although total white cell count was normal. Chemistry showed an increased total bilirubin [1mg/dL (0-0.2)], likely due to increased breakdown products from red blood cells. The indirect, or unconjugated bilirubin [0.8 mg/dL (0-0.2)], was much greater than the direct [0.2 mg/dL (0-0.1)] as seen in acute hemolytic anemia.

Pip was negative for FeLV, FIV, and exhibited a low positive titer indicating exposure to FIP.

Mycoplasma haemofelis PCR assay was positive for the presence of the organism in blood.

Pip's urinalysis revealed bilirubinuria (Bilirubin – 6 mg/dL, Crystals – moderate bilirubin) likely due to hemolytic anemia (Hemoprotein – trace), and showed no red blood cells

to indicate urinary blood loss. Giardia was found on fecal examination, but no melena was noted to indicate gastrointestinal blood loss. The Giardia, therefore, was interpreted to be unrelated to his clinical signs, although it may have been involved in immunosuppression leading to the clinical manifestation of FHM.

Worsening clinical signs and serial QATs revealed that Pip was in the middle of a hemolytic crisis (PCV 16%, mildly icteric serum at 8am, PCV 14%, moderately icteric at 2pm).

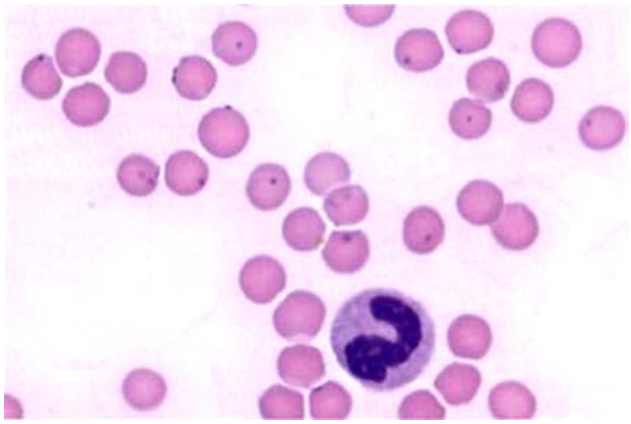
Diagnosis: Feline Hemotropic Mycoplasmosis

Treatment: Pip received the treatment of choice for an acute hemolytic crisis due to FHM.[1,2,3] Since his PCV dropped below 15%, Pip received 1 unit of Type A packed red bloods cells at 2pm. He was also started on doxycycline (5 mg/kg IV BiD for 1 day) to treat the bacteremia and fenbendazole (200mg once daily for 7 days) for the Giardia. After the transfusion, Pip's PCV was 28%. At his next serial measurement (8am on 7/24/03), his PCV was 21%; he was having a transfusion reaction. Therefore, he was started on oral prednisolone (1-2 mg/kg PO BiD) to diminish the extravascular hemolysis of donor red blood cells and red blood cells affected by FHM. He was changed over to oral doxycycline that day at the same dose.

Outcome: On 7/25/03, just two days after initial presentation, Pip's PCV was 30%. There were no detectable organisms on blood smear and Pip showed no remaining clinical signs of disease. He was discharged to the care of his owner that afternoon. According to his owner, Pip has shown no signs of illness since he was discharged.

Discussion

Accurate timely diagnosis of a hemolytic crisis due to FHM in the clinical setting remains dependent on evaluation of blood smear despite the advent of new technologies (clinically available PCR assays require days to produce results).[1,2] Further, additional time can be saved (and earlier treatment can be instituted) if veterinarians and veterinary technicians are capable of in-house blood parasite analysis on blood smear. In the past, private practice veterinarians and other investigators have experienced high levels of frustration in the differentiation of red blood cell parasites (Figure 3).[1] In addition, immature red blood cells seen in strongly regenerative anemias have been erroneously identified as red blood cell organisms in many instances (Figure 4). Subsequently, it is of paramount importance that veterinarians and veterinary technicians are given ample training in the identification of these organisms.

a. 

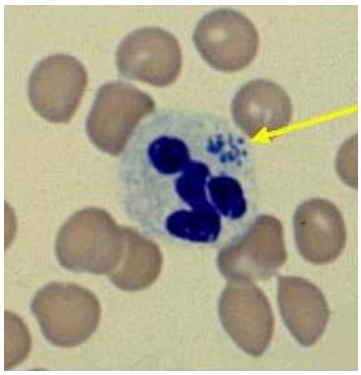
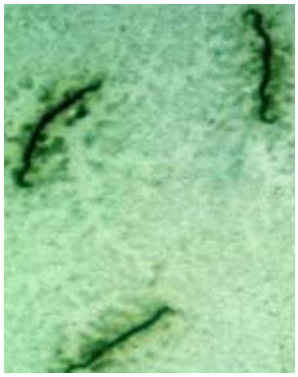
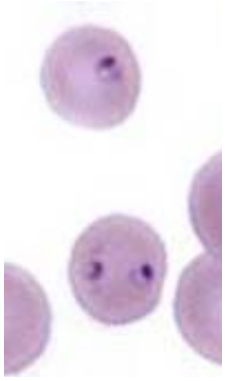
b.  c.  d.  e. 

Figure 3. There are several organisms that can be found on feline blood smears that can cause anemia. Each of these organisms is morphologically distinct, which aids in diagnosis.[4] On the differential list are

a. Mycoplasma haemofelis, 0.3um - 0.8um diameter epierythrocytic coccoid, rod-like, or ring-shaped organisms, some in short chains. Often affects many (up to 95%) of erythrocytes during acute infection.

b. Ehrlichia, granulocytic cytoplasmic inclusions of variable size in platelets or white blood cells, depending on species.

c. Babesia, 1-2.5 um cytoplasmic paired piriform structures within erythrocytes.

d. Leptospirosis, spiral and extracellular

e. Cytauxzoon felis. This protozoan is recognized as small 0.5 to 1.5 um cytoplasmic signet ring or safety pin-shaped bodies in red cells. Morphologically, it looks similar to Mycoplasma haemofelis, since the organisms are small and have a ring shape. However, usually less than 5% of red cells contain the parasite and the anemia is usually normocytic, normochromic due to the peracute nature of the disease. In addition, the clinical picture is very different. The presenting complaint is usually sudden death, and a vasculopathy, not the parasitemia, is the cause of death.[6]

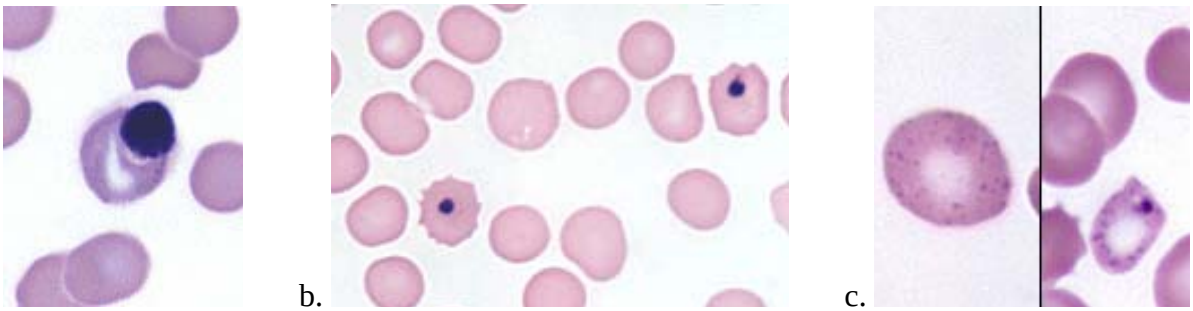


Figure 4. Immature Red Blood Cell Morphology as Sources of Error in Regenerative Anemias due to Blood Parasites.[7]
a. metarubricytes are nucleated red blood cells; b. Howell Jolly Bodies are red blood cells with nuclear remnants in the cytoplasm; c. basophilic stippling is due to RNA aggregates. These findings represent immature red blood cells that were released prematurely from the bone marrow due to a strong regenerative response. It is believed that blood parasites, especially *Mycoplasma haemofelis*, may be over-diagnosed because of misinterpretation of a strongly regenerative bone marrow response. Another source of error is stain precipitate on the slide.

It is also important to note that sometimes, even if organism is present, it will not be observed on blood smear. A negative smear does not rule out blood parasites. In this instance, or when identification of the organism using morphology proves difficult, additional tests including cultures, serologic tests, and PCR assays can be run for various organisms, although results will not be immediate.[1,2]

Conclusion

While the dawn of new technologies is beginning to illuminate the mysteries of Feline Hemotropic Mycoplasmosis, diagnosis in the clinical setting is still largely dependent on immediate blood smear evaluation. Currently, however, PCR is very useful in confirming diagnoses made by evaluation of blood smear. In addition, accurate identification of a sole organism on smear allows investigators to thoroughly characterize the specific organism's variable morphology, aiding in the creation of guidelines for diagnostic blood smear evaluation.

Also, it is very useful in epidemiologic studies, giving a definitive diagnosis of FHM rather than a presumptive diagnosis based on smear. Finally, as PCR technology becomes more popular and cheaper over time, it will likely become a readily available, highly accurate, and far superior definitive diagnostic tool for various infectious agents such as *Mycoplasma haemofelis* and *Mycoplasma haemominutum*.

References:
[1] Sykes J. E.: Feline Hemotropic Mycoplasmosis (feline hemobartonellosis). *Vet. Clin. North Am. (Small Anim. Pract.) 33* (4):773-787; 2003.
[2] Messick J. B.: New Perspectives about Hemotrophic mycoplasma (formerly, Haemobartonella and Eperythrozoon species) infections in dogs and cats. *Vet. Clin. North Am. (Small Anim. Pract.) 33* (6):1453-1465; 2003.
[3] Cowgill E. S., Latimer K. S.: Haemobartonellosis in Cats. *www.vet.uga.edu/vpp/clerk/Cowgill/Index.htm*: 1-7; 2001.
[4]Aird B., Tvedten H., Weiss D., Gaunt S.D.: Section III: The Erythrocytes. *Schalm's Veterinary Hematology*, 5th Ed. (Feldman B. V., Zinkl J. G., Jain N. C., Scalm O. W., eds.). Lippincott Williams & Wilkins, 2000; pp 140-161.
[5] Center S. A. Personal Lecture Notes: Renal and Hepatic Notes; 2002.
[6] Daily F. D., Rakich P. M., Latimer K. S.: Cytauxzoonosis in Cats: An Overview. *www.vet.uga.edu/vpp/clerk/Dailey*: 1-7; 2001.
[7] French T. W., Blue J. T., Stokol T.: Clinical Pathology Modules: Red Cell Morphology. *http://drylab1.vet.cornell.edu/ClinPathModules/*; 1997.